Cross
OCC

MODERN MICROSCOPY
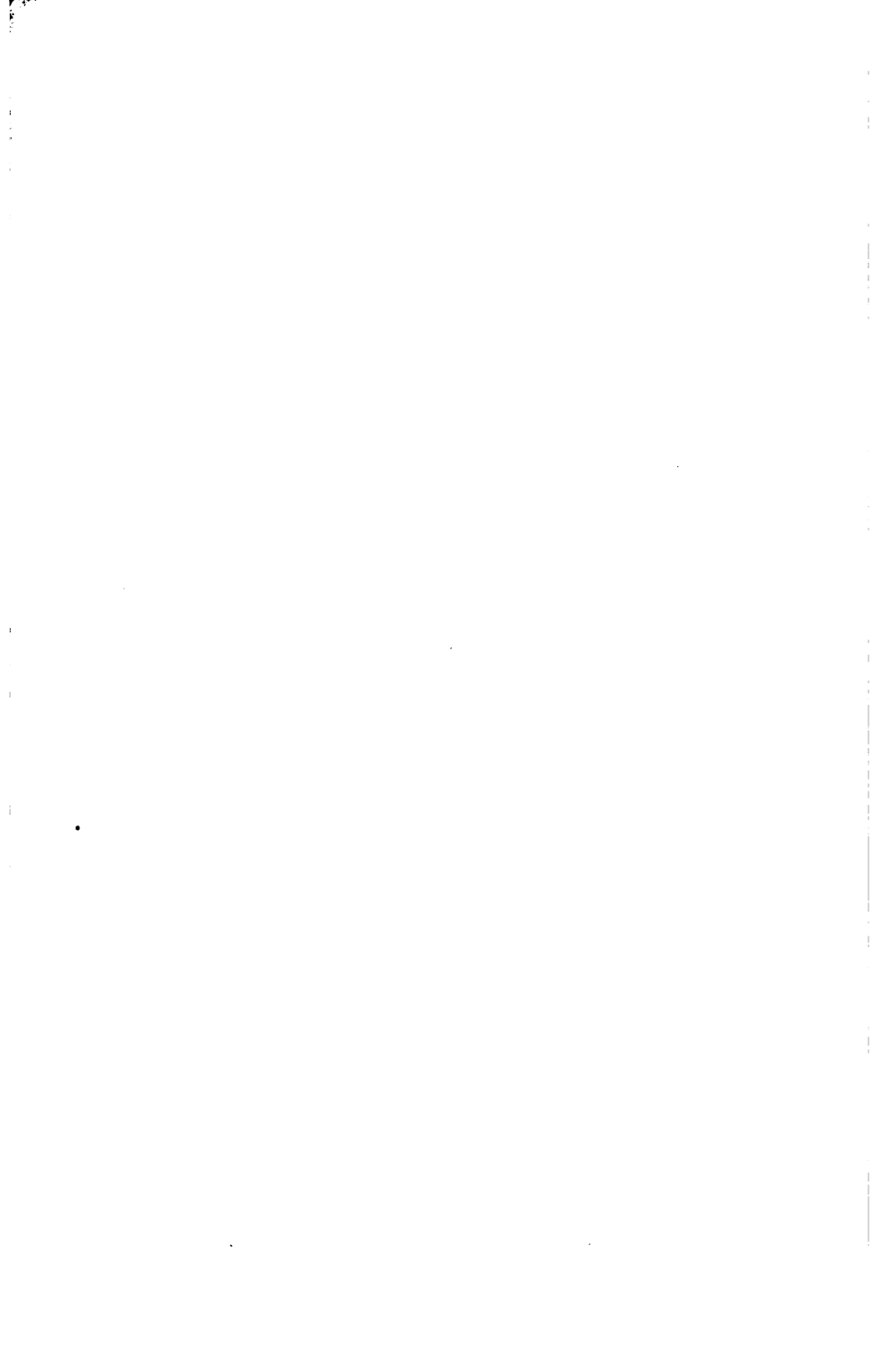
PLATE I.
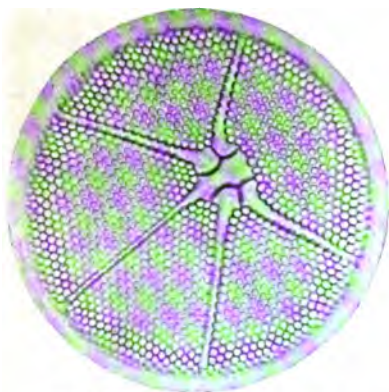
(A) ASTEROMPHALUS ARACHNE.
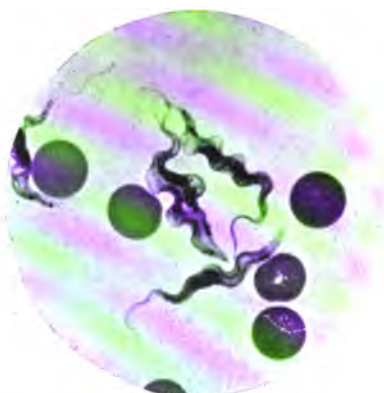
(B) TRYPANOSOME BRUCEI.
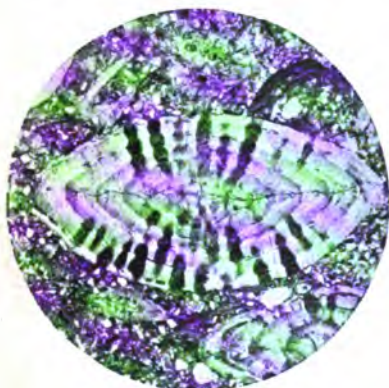
(C) SECTION OF NUMMULITE.
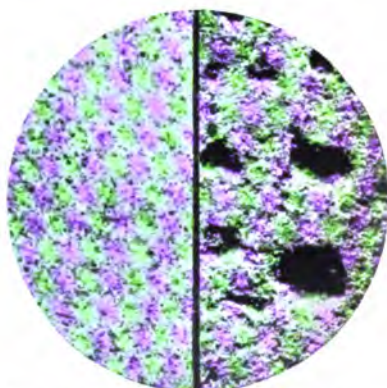
(D) CEMENT SURFACES FOR COMPARISON.
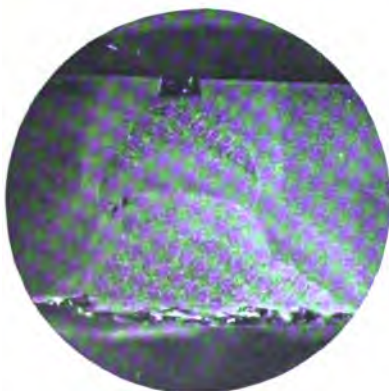
(E) STRUCTURE OF ELECTRIC WELD.
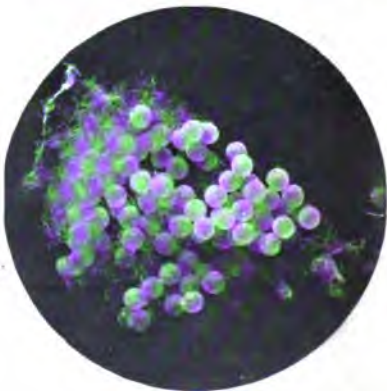
(F) EGGS OF SPIDER.

All the above by Members of the Photomicrographic Society.

(*See pp. 89 and 90.*)

Frontispiece

MODERN MICROSCOPY

A HANDBOOK FOR BEGINNERS AND STUDENTS

BY

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AND

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FIFTH EDITION

REVISED AND REARRANGED BY

HERBERT F. ANGUS

WITH CHAPTERS ON SPECIAL SUBJECTS BY VARIOUS WRITERS

CHICAGO

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PREFACE TO FIFTH EDITION

In the preparation of this new edition the original intention that it should be for beginners and students has been steadily kept in view.

The first part has been entirely rewritten to bring the book into line with present-day knowledge and methods, and Parts II. and III. have been added to and rearranged with the help of numerous friends and contributors, to whom our most cordial thanks are due, so as to present to the reader as complete a picture as possible, in the space available, of the Microscope in two widely dissimilar aspects—viz., as the handmaid to Science and as the key to a new and, for many, unsuspected world full of interest and beauty.

M. I. C.

London,
April, 1922.

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PREFACE TO FIRST EDITION

THIS handbook is not intended to be an exhaustive treatise on the microscope, nor to give particulars of the various patterns of instruments that are made, of which details can be seen in the makers' catalogues, but to afford such information and advice as will assist the novice in choosing his microscope and accessories, and direct him in his initial acquaintance with the way to use it.

The directions for preparing microscopic objects by Mr. Martin J. Cole are the outcome of a very long experience as a preparer of microscopic objects of the highest class, and cannot fail to be of the greatest service to the working microscopist.

M. I. CROSS.

LONDON, 1898.

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PART I

THE MICROSCOPE—ITS CONSTRUCTION AND USE

PART II

THE MICROSCOPE AND THE SCIENTIST

CONTENTS

PART III

THE MICROSCOPE AND THE NATURALIST

INTRODUCTION

THE development of the microscope to its present state of perfection has been contemporaneous with the growth of scientific knowledge during the past century. It was only in 1824 that Tully made his first Achromatic Microscope, and the records of Medical Research, the Royal Microscopical and other Societies, and numerous departments of industry, bear witness to the unceasing efforts that have been made to improve the optical portion, and to render the mechanical means for using the lenses convenient and efficient for the purpose. As the handmaid of science, it has always kept pace with the needs of the workers in the new avenues which have opened up, frequently making a stride forward, either in theory or construction, and assisting in the elucidation of new facts or the clearer perception of fine structure.

The assured position of the microscope in relation to scientific work will be evident by reference to the second part of this book, but microscopy has a wider sphere as a minister to pleasure and recreation. In this capacity it offers to its users the power to see into a world teeming with life that is invisible to the unaided eye. It is unfortunate that the interest of the amateur is less active than formerly, for much of the improvement in the microscope has been due to the criticism and encouragement of amateur microscopists. The third part of this book will indicate directions in which profitable work may be done by any intelligent observer.

It is not possible, without instruction and considerable practice, to make the best use of the microscope, and it is only by knowing fully what can be done and how to do it that the worker will be enabled to wrest from his instrument all that it is capable of yielding.

There is a tendency for work to be less well done than it need be, through insufficient teaching and appreciation of elementary facts, and its value to the individual user depends very largely on the methods of manipulation adopted.

It is hoped that the directions given in this book will assist beginners and all who work with the microscope to a clear understanding of the best way of using their instruments.

The due presentation of Parts II. and III. has somewhat encroached on the space originally allotted to Part I., but nothing essential has been omitted, and, studied in conjunction with the various makers' catalogues, it is hoped that a more complete understanding will be obtained of the construction and use of the instrument than if more space had been allotted to the subject in an endeavour to make it complete in itself.

The four makers whose catalogues should be consulted are :

Charles Baker, 244, High Holborn, London, W.C. 1.

R. and J. Beck, Ltd., 68, Cornhill, London, E.C. 3.

J. Swift and Son, Ltd., 81, Tottenham Court Road, London, W. 1.

W. Watson and Sons, Ltd., 313, High Holborn, London, W.C. 1.

MODERN MICROSCOPY

PART I

DEFINITIONS

Glass—its Physical Properties.

MANY people think of a microscope as a more or less complex
mechanism of brass, beautifully lacquered, to which one or two
lenses are incidentally attached, but actually the lenses are the
essential part, the mechanism being only incidental.

These lenses might be made of any transparent substance,
which could be worked with accuracy to predetermined curves,
and in bygone days, when the glass-makers' art had not reached
the degree of refinement it has to-day, attempts were made to
use natural crystals, including precious stones ; but the varieties
of glass now obtainable render recourse to such material un-
necessary, excepting perhaps in the case of fluorite, which in
some optical combinations gives a correction as yet unobtainable
with any variety of artificial glass, and quartz, which must be
used when it is essential (see Chapter XII.) that ultra-violet light
should be employed, as in some research work.

It is common knowledge that a convex lens increases the
apparent size when the object looked at and the eye are placed
in a correct position relative to it.   This it does by altering the
path of the light rays passing through it in accordance with the
law of refraction (Fig. 1).

But it is not generally known that the apparent size of the
object, due primarily to the curved surfaces of the lens, is also
influenced by the density of the glass—that is to say, that two

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lenses having the same curvature, but of different densities, would not give images of equal size: the lens made from the denser glass would refract or bend the light rays more, and the image would be larger.

The numerical expression of this bending power as compared with air, taken as unity, is called the refractive index, or R. 1.
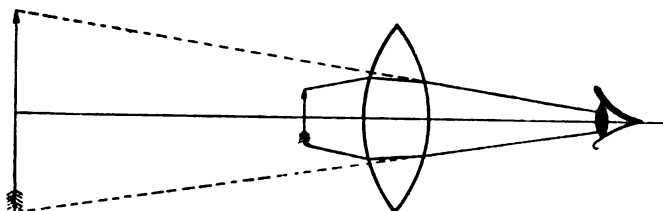
FIG. 1.—ENLARGED IMAGE OF AN OBJECT SEEN THROUGH A BI-CONVEX LENS.

The refractive index of water is 1·33, while that of glass ranges from 1·4785 to 1·7566.

It is also generally known that the various colours occurring in nature indicate as many different wave-lengths of the vibrations which constitute light. These colours or vibrations of varying wave-lengths are unequally affected when passing through
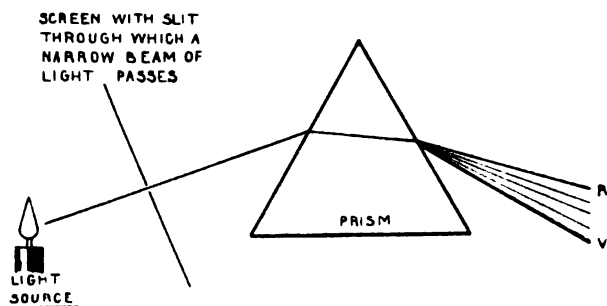
FIG. 2.—BEAM OF LIGHT SPLIT UP INTO ITS COMPONENT COLOURS BY MEANS OF A PRISM.

a lens, but the effect is especially noticeable when a well-defined beam of light is passed through a prism, as diagram (Fig. 2). It is also observable when looking through a powerful single lens, such as a burning-glass or a cheap field-glass, the object being fringed with colour: the more powerful the uncorrected lens the more marked the colour.

This is known as dispersion, and, like density or power of refraction, is another variable property of glass altogether independent of the density. It would be possible, therefore, to have two lenses of the same curvature and the same density or refractive power, one of which would give a more pronounced colour fringe to the image than the other.

Hence arises the possibility of constructing a complete range of special glasses known as optical glass, which, by the admixture of minute quantities of various elements, can be made to give varying proportions of refractive and dispersive power. With such a series of glasses it is possible to correct the faults of one lens, or series of lenses, by another or second series, or even a third. See Figs. 7, 8, and 9, p. 8, for the means of correction; and Fig. 28, p. 23, for one of the chief faults which, in addition to that noted above, must be corrected.

Magnification.

To examine an object in which there is fine structure one instinctively approaches it and, if it is portable, lifts it up. The nearer it is brought to the eye the larger the image on the retina and the more visible the fine detail becomes, until a point is reached where the internal lens of the eye, which automatically adjusts itself for objects at different distances, is at its extreme limit of adjustment. For the greater part of adult life this limit for the normal eye is set at 10 inches, and if an object is brought closer than this, the gain in size of the image is counterbalanced by the loss of definition.

The Standard of Comparison.

This, then, is the standard of comparison when speaking of magnification, the maximum size of image obtainable with the unaided eye, and on this the nomenclature of microscope lenses is based.

If it were possible to see a small disc sharply defined at 1 inch distance from the eye, it would be found ten times the width of the same disc held at 10 inches, the apparent diameter of an object being proportionate to the distance from the eye. A lens, therefore, which will give at 10 inches on an optical bench an image ten times the width of the object upon which it is focussed

is called a 1 inch (a lens of 1 inch focus producing approximately this effect), and is said to magnify 10 diameters, or simply 10 × . Similarly, one giving 20 × is called ½ inch, 60 × ⅓ inch, and so on throughout the whole range.

Diffraction and the Abbe Theory.

Magnification looked at from this point of view is simple enough, but it was found, before the present-day theory of microscopical vision was propounded, that lenses giving the same magnification did not necessarily show the same amount of detail, or, to use the microscopical expression, give the same resolution.

This remained a puzzle until Professor Abbe solved the problem by pointing out that the very fine detail of microscopic objects, acting as a grating, sets up interference phenomena, the vibrations of light damping one another out in such a way that, having passed the object, the rays, no longer vibrating in every azimuth and at all angles of inclination, are broken up into a central beam and a number of subsidiary or diffracted beams spaced, according to the nature of the structure, about the central beam in various patterns, which are repeated at definite distances from the central beam outwards, according to the fineness of the structure under examination. The finer the structure the greater the distance of the primary series of diffracted beams from the centre and the greater the spacing between the primary, secondary, and succeeding spectra, as the diffracted beams are called, the light being broken up in the process so that each diffracted beam appears as a miniature spectrum.

He proved by a simple series of diaphragms that, unless a lens could receive light at a sufficiently wide angle to take in at least a portion of the first diffracted beam, as well as the central beam, no detail could be observed in the object, and that the greater the number of spectra received by the lens, the greater the accuracy of the image, although for all practical purposes the primary, or even a portion of the primary, was sufficient to give correct information as to the number of lines or dots in any given structure, but not necessarily the correct relative spacing of such detail.

Numerical Aperture.

Professor Abbe also suggested a method of expressing numerically the resolving power of a lens, whether used dry or with the front immersed.

It had already been observed that the elimination of the air space between the lens front and the thin glass covering the object by the interposition of a fluid medium gave increased resolution, owing to the reflection and refraction of the light rays at the surface of the cover-glass being avoided; but the importance of thus passing on to the lens the rays making the

COVER GLASS

UPPER SURFACE
OF OBJECT SLIP

DRY   WATER   OIL
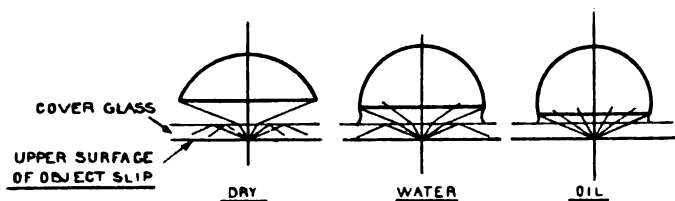
FIG. 3.—LENSES WITH FRONTS DRY AND IMMERSED, SHOWING RAYS RECEIVED
BY EACH.

greatest angle with the central beam was not understood until Professor Abbe pointed out that these wide angle rays alone resolved the detail of the very finest structures.

There was no accepted method of comparing the performance of dry and immersed lenses, and the whole question was confused until Professor Abbe suggested compounding the angle of light which the lens passed (angular aperture) with the medium in which the lens was used by multiplying the sine of half the angle of aperture by the refractive index of the medium; this measure of the resolving power of an objective, known as 'numerical aperture,' is usually abbreviated to N.A.

Optical Index.*

Obviously the N.A. and magnification must bear some intimate relation, as it would be equally useless to combine high magnification with too low an N.A., or high N.A. with a magnification so low as to leave the detail resolved still invisible; the extremes

* Owing to the use of unavoidable technicalities this definition will hardly be intelligible to the beginner until he has digested the information given in Chapters II. and III.

are useless, and even a marked tendency in either direction renders manipulation extremely difficult.

Nelson, with whom the study of the possibilities of the microscope has been a life's work, suggested, therefore, another less well-known but valuable term, called the 'optical index,' abbreviated to O.I., which defines the N.A. necessary for every 100 × of magnification (10 initial × 10 eyepiece) as N.A. 0·26, expressed numerically by multiplying the N.A. by 1,000 and dividing by the initial magnification. The O.I. therefore of the ideal lens is 26, and the nearer the O.I. of any lens approaches this figure the greater its efficiency; but this is the limit when the microscope is used by an expert under the most favourable conditions. In practice, for routine work, in the execution of which it is impossible to exercise the same care, the ratio of magnification to N.A. is increased in the proportion of approximately 2 : 1.

Thus a lens of initial magnification 50 on the long tube that is $\frac{1}{6}$ inch would supply, used in conjunction with an eyepiece 10 ×, all the magnification required to utilize the resolving power of N.A. 1·30 which is the N.A. usually associated with a $\frac{1}{12}$ inch.

The O.I. of such a lens would be:

$$\frac{1·30 \times 1,000}{50} = 26,$$

which corresponds exactly with the definition given above.

Allowing for difference of tube length, which necessitates a somewhat higher initial magnification to compensate for the shorter tube now almost universally used, a $\frac{1}{7}$ inch of N.A. 1·30 would approximate very closely to it, which does but confirm the view expressed by Barnard on p. 95 under subhead 'Diagnostic Apparatus.'

For minor definitions see Glossary of Technical Terms, p. 78.

OPTICAL ELEMENTS

Magnifiers, Objectives, Eyepieces, Tube-Length.

In the preceding chapter we purposely used the more general term 'lens' to designate the instrument of magnification. We must now differentiate between those low-power lenses, which are used by themselves alone, usually called 'magnifiers,' and those which form part of an optical system.

These **Magnifiers** may be simple or compound.
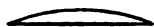
FIG. 4.—SIMPLE MAGNIFIER (PLANO-CONVEX).   FIG. 5.—COMPOUND MAGNIFIER (APLANATIC).   FIG. 6.—SIMPLE MAGNIFIER (BI-CONVEX).

The simple magnifier, as indicated in above diagram (Figs. 4 and 6) is merely one piece of glass ground to a convex curve on one or both sides. The usefulness of such a lens is limited to approximately 2-inch 5×. In higher powers the want of flatness in the field of view, due to spherical aberration (see Glossary of Technical Terms, p. 78, and diagram illustrating this fault, Fig. 28, p. 23), also colour fringes round the object, become very marked.

The most usual form of compound magnifier is composed of three lenses cemented together after Steinheil's formula (Fig. 5). The corrections thus introduced enable one to use this magnifier up to a power of ½-inch 20× or even ⅓-inch 30× ; but the field of view in the latter is very limited, and the working distance very small. These lenses are known as Aplanats.

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The usual powers in this type are :

<div align="center">

6 ×   10 ×   12 ×   16 ×   20 ×.

</div>

The image given by both these types of magnifier is erect
(see Fig. 1, p. 2) — that is to say, the image is seen the
same way up as the object, and the movement of any tool used
while the object is being manipulated is not reversed ; but, as
already indicated, the higher powers are difficult to use owing
to close working distance, close eyepoint, and limitation of field
of view.

All these disadvantages can be overcome, however, by picking
up the image with another magnifier, called an ' eyepiece,' and a
lens used in this way with an eyepiece is called an ' objective.'

Objectives.

Owing to the fact that the image given by an objective is
magnified by an eyepiece, it must be still better corrected.   This
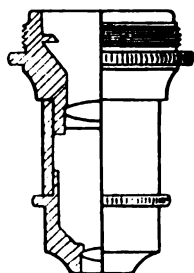
FIG. 7.—LOW
POWER.
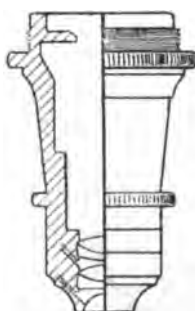
FIG. 8.—MEDIUM
POWER.
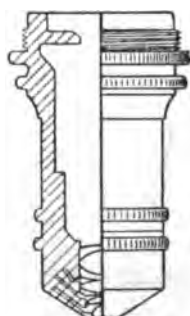
FIG. 9.—HIGH
POWER.

is effected by mounting one, two, or three combinations behind
the front lens, which may itself be simple, as in Figs. 8 and
9, or compound, as in Fig. 7, all accurately centred with it,
every detail of these additional lenses—the glasses of which they
are composed, the curves to which they are ground, and the
distances apart at which they are set—being carefully computed
to correct the faults in the image given by the front lens, which
latter is chiefly responsible for the magnification, before it is
passed on to the eyepiece.

In the accompanying diagram the train of lenses required for the correction of a low, medium, and high-power objective are shown, each element of the train usually consisting of two simple lenses cemented together, forming a so-called doublet.

Eyepieces or Oculars.

Eyepieces used in conjunction with above magnify the initial image given by the objective from $5 \times$ to $20 \times$. Higher powers are sometimes used for testing and comparing the performance of objectives. They are, however, testing appliances rather than parts of a microscope, however complete, and as such would be out of place if included here.

Many patterns have been devised from time to time. There are, however, only three in general use, as figured below. Of
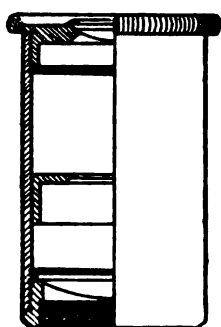
FIG. 10.
HUYGHENIAN.
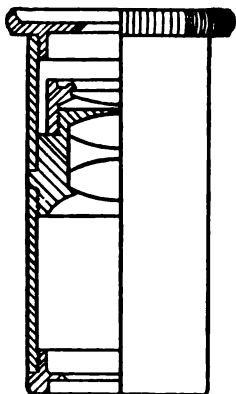
FIG. 11.
COMPENSATING.
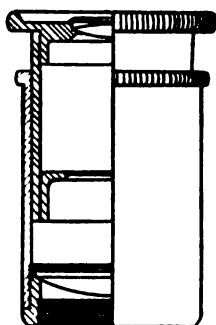
FIG. 12.
ADJUSTABLE.

these, the Huyghenian is the type supplied with 99 per cent. of present-day outfits, the most useful magnifications being $6 \times$ and $10 \times$; but the other two types are extremely useful in research work, more particularly in the higher powers (see Chapter IX.).

Tube-Length.

As the total magnification of the objective and eyepiece is dependent on the distance separating them, its influence must now be considered.

We have seen in Chapter I. that a magnified image is always compared with the size of the object, as seen by the unaided eye, at the nearest point at which distinct vision can be maintained—viz., 10 inches—the degree of magnification being thus determined.

Based on this standard, the initial power, or power before applying the eyepiece of an objective 1 inch, is $10 \times$, and if the eyepiece is so adjusted as to pick up the image at this point, the power of the eyepiece being $10 \times$, the total magnification will be $100 \times$.

The earlier microscopes were constructed on this principle, the distance between objective and eyepiece being 10 inches, but the extended use of the instrument by students created a demand for a more portable form, resulting in the reduction of the length of tube to approximately $6\frac{1}{2}$ inches, or two-thirds of the standard. Therefore the initial magnification of an objective 1 inch on such a microscope is $10 \times \frac{2}{3} = 6\frac{2}{3}$, and the total magnification with an eyepiece $10 \times$ is $66 \times$.

For all ordinary purposes the tube-length may be reckoned from the two ends of the adjustable tube, into one end of which the objective screws, and upon the other end of which the eyepiece rests ; but for very critical work, comparison of performance of objectives, etc., a more accurate method must be employed, as the higher power objectives can only be used efficiently at the exact optical tube-length for which they have been adjusted in the course of manufacture, after an allowance has been made for possible variation of conditions (see Chapter XI.).

CHAPTER III

OPTICAL SYSTEMS

Erect and Inverted Image: Monocular and Binocular.

As we have seen in the foregoing chapter, the addition of an eyepiece increases working distance and power, but the image seen by means of such an optical system, owing to the crossing of the rays within it, becomes inverted. This is no disadvantage in observation microscopes, but the erect image is essential for
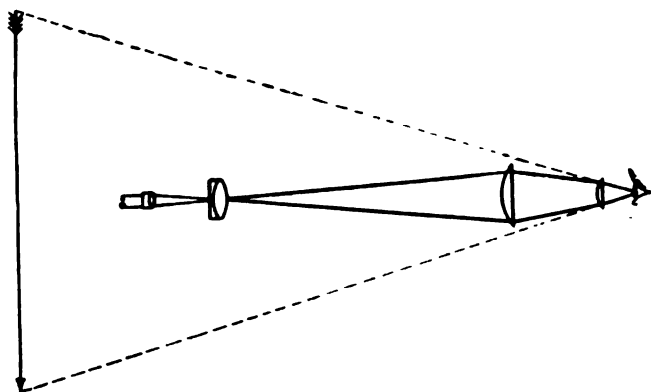
FIG. 13.

selecting, preparing, and mounting specimens for subsequent examination with the observation microscope, as all movements of the necessary tools are reversed if the image is inverted.

Erectors.

The inverted image can, however, be reinverted or erected by interposing a series of reflecting surfaces between the objective

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and the eyepiece.   The two most satisfactory methods of doing
this are shown in the accompanying diagrams.

Both are efficient, the train of Porro prisms (Fig. 15),
similar to those used in a prismatic field-glass, as suggested by
Greenough, being more generally used, possibly on account of
the pronounced stereoscopic effect obtained, by means of paired
objectives, when used binocularly, and to the simplicity of the
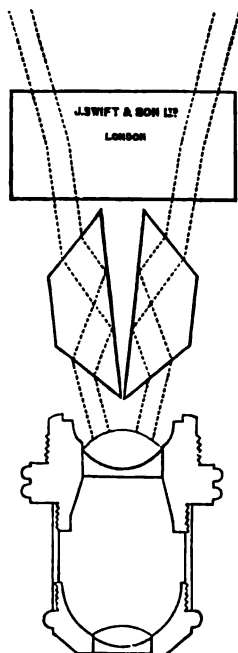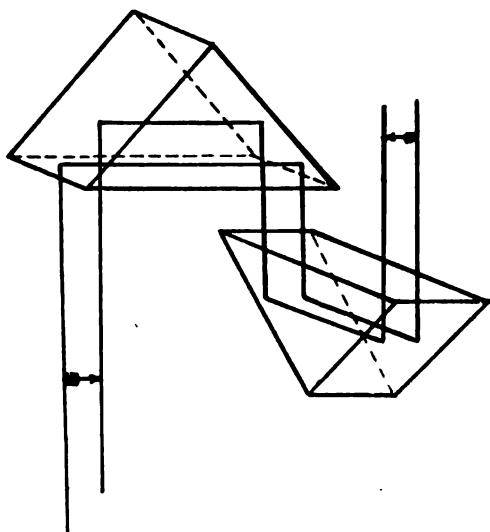
FIG. 14.   FIG. 15.

monocular form, which can be had merely as an attachment;
but the Stephenson (Fig. 14) is far superior for prolonged work,
because it not only erects the image, but enables the body of the
microscope to be set at an angle in relation to the object.

It is generally necessary to keep an object in course of pre-
paration horizontal, and the possibility of doing so while looking
down a tube at a convenient angle will be appreciated by all
who have attempted exacting work of a fine nature for a
prolonged period (see p. 263).

In the Porro type of erector the tube must be at right angles
to the object stage.

Erect Image Systems.

We have, therefore, available for preparation microscopes, two types of magnifier used without eyepiece, and two types of an objective-eyepiece system with interposed erector—viz. :

(1) Simple magnifier without eyepiece, as Figs. 4 and 6.

(2) Compound magnifier without eyepiece, as Fig. 5.

(3) Stephenson with eyepiece and erector, as Fig. 14.

(4) Porro-Greenough with eyepiece and erector, as Fig. 15.

The maximum useful magnification of No. 1 is 5×, of No. 2 20×, of No. 4 100×, while that of No. 3 is limited only by the working distance of the objective—that is, the distance between the front lens and the object—which must, however, be sufficient to allow of the necessary manipulation.

Nos. 3 and 4 can be had either monocular, which is unusual, or binocular.

A series of illustrations showing the usual method of mounting each of the above will be found in Chapter VI.

Inverted Image Systems.

Observation microscopes are all of this type; they may be monocular, as is the case with 99 per cent. of the instruments in use, or binocular.

MONOCULAR.

The practical limitation of magnification of the monocular form of this type is 1,000×, such magnification being made effectively possible by the immersion of the front of the objective in a medium (known as cedar-wood or immersion oil) of the same refractive index as an average crown glass, 1·52.

The most useful objectives are: 2 inch, 1 inch, ⅔ inch, ⅙ inch, used dry, and $\frac{1}{12}$ inch used immersed in oil; although weaker, intermediate, and even higher powers are made, the last, however, present no advantage in resolution, and little, if any, in magnification.

These, with the two most useful Huyghenian eyepieces, 6× and 10×, give on the 6⅔-inch tube a range of magnifications as under :

			2 in.	1 in.	⅔ in.	⅙ in.	$\frac{1}{12}$ in.
Eyepiece	6×	...	20×	40×	60×	240×	480×
,,	10×	...	33×	66×	100×	400×	800×

The highest objective, although universally called a $\frac{1}{12}$ inch, is about $\frac{1}{14}$ inch by actual measurement; consequently, the magnifications obtained are somewhat higher than those shown in this table, which are calculated for a true $\frac{1}{12}$ inch, thus bringing the total maximum magnification up to $1,000\times$, as referred to repeatedly throughout these pages.

BINOCULAR.

Of the binocular observation microscopes there two types: the Wenham, which is the older and less efficient, seeing that it is useless as a binocular for powers exceeding 200 to 300 ×, and
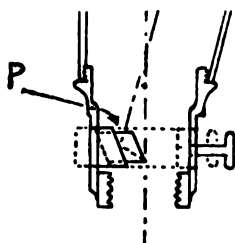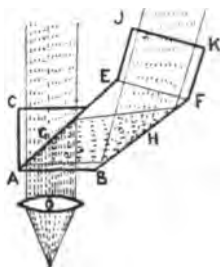
FIG. 16.   FIG. 17.

the comparatively recent high-power binocular, which, as its name implies, has no such limitation.

The above diagrams show the means employed in either pattern to split up the light after it has passed through the objective into two beams.

In the Wenham (Fig. 16) half the beam passes direct to the eye, the other half being diverted by means of the prism P, the edge of which bisects the back lens of the objective. In the more modern pattern (Fig. 17) the separation is effected by a lightly silvered reflecting surface, which allows the whole beam to pass directly through it (the prism G being added to maintain direction), reduced in intensity by one-half, the remaining half being reflected from the silvered surface to the second tube, up which it passes, after being again reflected at H.

Both patterns can be used as a monocular by sliding the prism out of the optic axis of the instrument.

To these two types may be added the Greenough, described in

the earlier pages of this chapter as a preparation microscope, owing to the fact that it gives an erect image; but it is often used as an observation instrument, on account of the wonderful plasticity of the image obtained with it.

Therefore we have:

For objects requiring a magnification of not more than $100 \times$, such as botanical and entomological objects and the larger forms of pond life, the Greenough.

For the above, together with the whole range of pond life, the Wenham (see p. 235).

For objects requiring greater magnification than 200 to $300 \times$, the high-power binocular.

ILLUMINATION—GENERAL

For Opaque and Transparent Objects.

HITHERTO we have presupposed the efficient illumination of the object examined, for the magnifications specified would be quite unusable if such were not the case.

We will now examine the means available for illuminating an object, firstly as regards the source of the light, and secondly as to the optical equipment necessary to utilize it to the best advantage.

The Source of Light.

This may be natural or artificial. If a north window were available looking out on to a huge cumulus cloud brilliantly lighted by the summer sun, and it were possible for these conditions to be constant whenever the microscope was in use, nothing more would be required, but unfortunately such is not the case. An artificial source is therefore to be preferred.

Of artificial sources of light there are in these days an almost embarrassing variety, and one of the best, if not the best, for all-round research work is still an oil lamp; yet, if the best, it is certainly not the most convenient, and it is necessary, when recommending appliances, to draw a sharp distinction between the microscope used as an instrument for investigating the unknown and the microscope used as a tool for routine work, such as demonstrating well-known histological detail to students, or for determining the presence or absence of certain organisms or constituents by which the medical officer of health, the engineer, or the chemist may be guided.

For the former, no refinement which will add a fraction of

1 per cent. to the efficiency of the outfit can be thought un-
necessary, but if, with misguided zeal, these refinements are
recommended to the routine worker, he is not helped, but merely
hampered thereby.

We have therefore devoted a chapter to the consideration of
artificial illuminants suitable for the research worker (see
Chapter VII.), and will proceed to lay down the requirements
for a suitable universal lamp for the routine worker, merely
remarking that, if the microscope so illuminated does not give
the best of which it is capable, the loss in efficiency is negligible
where known structure is concerned.

The essentials are : An electric lamp of, say, sixteen candle-
power with frosted bulb, adjustable for height throughout a
range of, say, 12 inches, the lowest point from the table to the
centre of the bulb being about 2½ inches, hooded in such a way
that no direct light reaches the eye, but yet allows a condensing
lens to be placed in close proximity to the bulb.

For the examination of transparent objects only the adjustment
for height could be dispensed with, but the above specification
will be found universally useful.

Having decided upon the source of light, we will now consider
how best it can be applied to illuminate efficiently an object
under examination.

From the illumination point of view objects are of two classes,
transparent and opaque, the former preponderating, but as the
illumination of an opaque object is the simpler problem, we will
take this class first.

Opaque Objects.

For objects requiring but low magnification, where there is
plenty of working distance—that is, distance between the front
of the objective and the object—a condensing lens, called a
bull's-eye, will usually be found sufficient.   Such lenses range
from 1½ to 2½ inches in diameter, and the usual method of mount-
ing is shown in Fig. 52a, p. 45.   Fig. 18, showing the path of
rays when the lens is placed at different distances from the light
source L, will enable one to adjust such a lens to better advantage
than pages of description.

For somewhat closer working distances—say ⅜ to ½ inch—or

2

where the greatest possible brilliance is required, a silver side
reflector used in conjunction with the bull's-eye will be found
useful.

The essential part of this reflector is a highly polished para-
boloidal surface cut out at the top in order that it may fit
closely to the objective, as shown in Fig. 19.
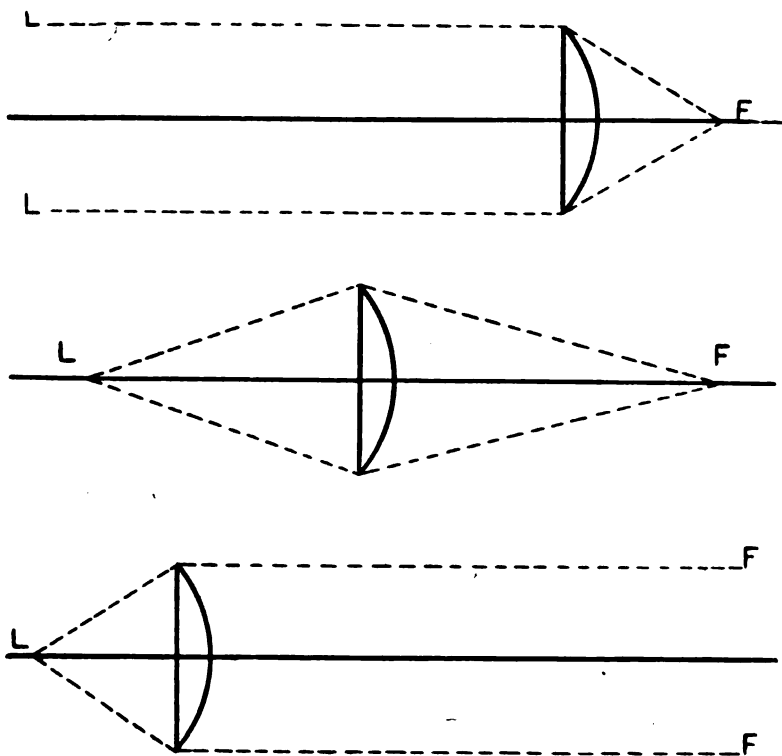
FIG. 18

Objects necessitating the use of objectives with still closer
working distance—say $\frac{1}{4}$ to $\frac{1}{6}$ inch—can often be efficiently
illuminated by using the condensing lens in the position shown
in Fig. 20, but this depends somewhat on the nature of the
object and the skill of the operator. As a general rule, such
objects are best illuminated by means of a vertical illuminator,
which is used in conjunction with the condensing lens.

There are two patterns of vertical illuminator, both dependent on the same idea—namely, that of using the objective itself as a condensing system, by means of which light is focussed upon the object, the magnified image of the object so illuminated being transmitted to the eyepiece through the same objective.

To do this it is necessary to interpose between the objective and the tube of the microscope a reflector, the so-called vertical
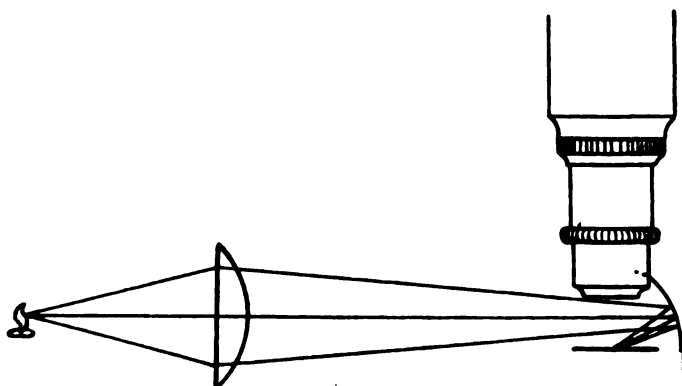
Fig 19.
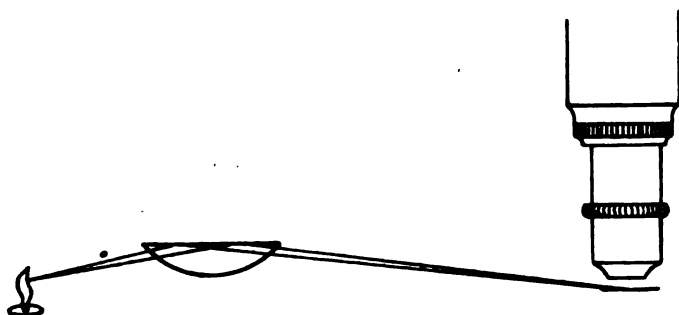
Fig. 20.

illuminator, which shall throw a beam of light, projected upon it by the condensing lens, downwards through the objective, as shown in diagram (Fig. 21), of which the reflector is a prism bisecting the objective, one half being used for illumination and the other for observation. In Fig. 22 the reflector consists of a plate of thin glass, the whole of the objective being used for condensing the light and also for observation.

For low powers this transparent reflector is sometimes placed between objective-front and object (see Chapter XVII.).
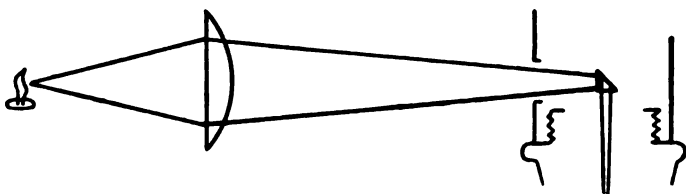
FIG. 21.
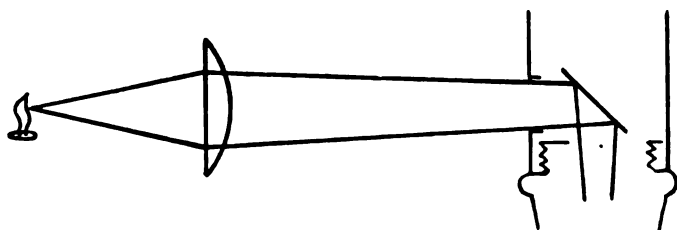
FIG. 22.

Transparent Objects.

These, as already stated, owing to the extreme tenuity of microscopic objects proper, and to the fact that the larger objects are cut or ground into slices, constitute the bulk of objects with which a microscopist has to deal.

Mirrors.—Every microscope, therefore, whether it be of the preparation or observation type, is fitted with a mirror or mirrors, usually two, of silvered glass, one plane and the other concave.

In the highest critical work, in which it is necessary to focus the image of the illuminant, or source of light, in the same plane as the object, the new stainless steel is sometimes used instead of silvered glass for the plane mirror, a first surface image being thus obtained free from duplication of image always present in a glass mirror of the ordinary type, often to such an extent, owing to want of parallelism between the two surfaces of the glass, as to render the mirror useless except for routine work.

White opal glass, or white card, is also useful either in place of, or mounted in a cap fitting over, the usual plane glass mirror of a preparation microscope.

The purpose of these mirrors or reflectors is to receive the light from the light source, and reflect it upwards parallel to and coincident with the optic axis of the instrument. The plane mirror does nothing more ; it merely alters the direction of the light rays without altering their character in any way. If parallel before, they remain parallel after reflection ; if divergent, divergent. It
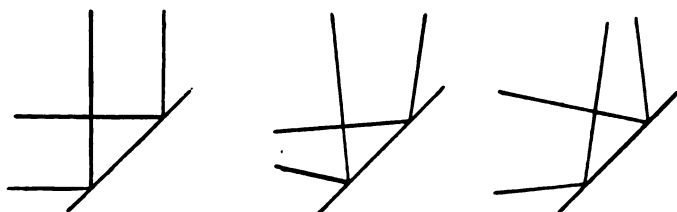
FIG. 23.

is immaterial, therefore, at what distance this mirror is fixed below the object, as shown in diagram, but the concave mirror condenses the light, bringing parallel rays, also slightly divergent and convergent rays, to a focus on the object, according to the distance at which it is set beneath it, as shown in diagram below.

In low-power microscopes, therefore, where no other means of concentrating light on the object is provided, this mirror should
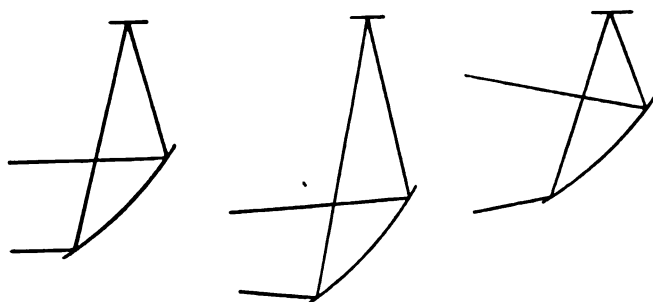
FIG. 24.

be adjustable as to distance from the object, but very few modern microscopes are so poorly equipped as not to have a sub-stage condenser, and, as the concave mirror is never used with any form of lenticular condenser, its retention, in all but the cheapest patterns, is somewhat of an anachronism.

Condensers.—The concave mirror as shown above concen-

trates light on the object, however inefficiently, but what the concave mirror does inefficiently a series of lenses used with the plane mirror will do far more efficiently, although it is as true of the lenses as of the mirror that they cannot increase the amount of light given by the illuminant; they can only concentrate it, which, however, the better class condensers do to such good purpose that a tinted glass screen is grateful to the eye, even when the source of light is only an oil lamp.

Optical Systems.—The chief types are shown below, from which it will be seen that the same method of correction is applied to these sub-stage systems as to objectives (see Figs. 7, 8, 9, p. 8); but the need of correction is not nearly so great in the case of the condenser, consequently the majority of the instruments in use are fitted with the Abbe type (Fig. 25),
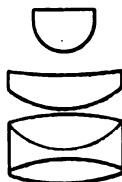
FIG. 25.—ABBE.   FIG. 26.   FIG. 27.—OIL
                       APLANATIC.   IMMERSION.

because it is inexpensive, easy to use, and gives reasonable efficiency for routine work—at any rate, up to the extreme limit of useful magnification, 1,000 ×.

Relative Efficiency.—It will not, however, give a sufficiently wide homogeneous or solid cone of light as to allow an objective over N.A. 0·70 to give the best result of which it would otherwise be capable; the reason is at once apparent if we trace the path of rays, axial and peripheral, through such a system (see Fig. 28). Owing to the spherical aberration, as it is called, one can only illuminate the object with a solid cone of light of N.A. 0·50, which is about the amount that an objective of N.A. 0·70 will pass without loss of definition (see p. 54), or with a wide angle hollow cone (Fig. 29), not with both; the focal planes of the marginal and central rays are so widely different that both cannot be brought coincident with the plane of the object at the same time.

For research work, therefore, an aplanatic (or achromatic, as it is sometimes called) condenser should be chosen (Fig. 26), or even one of the oil immersion type (Fig. 27) ; but such condensers require very careful adjustment to the optic axis of
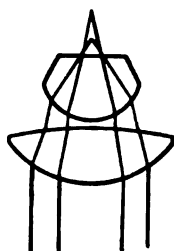
Fig. 28.

the microscope, which varies to an appreciable extent with each objective ; from which it follows that they can only be used to advantage on the finest stands.

The numerical apertures of these types are approximately as under :

Type.	Total Aperture.		Aplanatic Aperture.	
	Dry.	Immersed.	Dry.	Immersed.
Abbe	1·0	1·20	0·50	—
Aplanatic	1·0	—	0·90	—
Aplanatic, front lens removed	0·50	—	0·40	—
Immersion	—	1·80	—	1·80
Immersion, used dry	1·0	—	0·90	—
Immersion, front lens removed	0·60	—	0·55	—

All these condensers are usually fitted with an adjustable aperture diaphragm called an 'iris' diaphragm, by which the aperture can be varied at will—see p. 54 (3) ; also a carrier for stops, as Fig. 30, p. 26, or light modifers (see pp. 45, 46, and 97).

ILLUMINATION—SPECIAL

Monochromatic; Polarized; Dark-Ground.

The efficiency of the means of illuminating objects described in the preceding chapter can be increased for special work by—

1. Altering the character of the light itself.

2. Altering the homogeneousness of the beam, the character of the light remaining unaltered.

Monochromatic Illumination.

The character of the light can be altered by selecting vibrations of specified wave-lengths, or colour, and also by polarization.

The illumination of the object by light of approximately one wave-length, or colour, eliminates the chromatic aberration due to the dispersion of the glass used in making the objective (see Chapter I., pp. 2 and 3), which is never entirely corrected. This can be effected by using an illuminant giving only light of the particular wave-length required, by using an ordinary illuminant and stopping out by a light filter the wave-lengths not required, or by a combination of both methods.

The sharpening-up of the image thus effected is particularly useful in research work and photomicrography, but, as can readily be imagined, there is a very considerable loss of light involved whichever method be adopted, and the more nearly the light used approaches to a true monochrome the greater the loss. The fullest possible advantage of this method, therefore, can only be obtained by using a powerful illuminant, but for ordinary routine work a blue, or preferably green, glass screen, although it passes light throughout a series of wave-lengths other than the

dominant group which gives the glass its colour, will be found advantageous ; the reduction in intensity of illumination when used with an ordinary illuminant, such as described in the preceding chapter, being more than compensated for by the increased sharpness of the image.

Polarized Illumination.

The alteration of the character of the light by polarization is of primary importance to the geologist, as our knowledge of the constituents of the crystalline rocks is dependent on the differences of constitution revealed by this means.

For full explanation of what is meant by polarisation, of the means by which such illumination can be effected, and of the results thereby obtainable, see Chapter XVI. (' The Microscope in Geology ').

Dark-Ground Illumination.

The alteration of the homogeneousness of the illuminating beam without altering the character of the light itself is par-ticularly useful—in the lower powers to the naturalist, and in higher powers to the medical officer of health—as by such means it is possible to show the almost invisible transparent organisms of the pond and the altogether invisible disease germs —invisible, that is, by ordinary illumination in the living state —brilliantly illuminated in a field of inky blackness.

This is the so-called dark-ground, or dark-field, illumination ; it is effected for the

Low Power

objectives by stopping out the central beam of the condenser with an opaque disc.

Ordinary Condenser.—The stop (Fig. 30) for the Abbe should be half as wide again as the diaphragm opening, the edge of which is just visible at the back of the objective when the eyepiece is removed. This margin allows for stray light, want of perfect centration, influence of water when examining pond life., etc., and can be reduced in the better corrected condensers, more particularly if means are available for centring the fitting

carrying the condenser to the optic axis of the objective in use.

For very low powers the front lens of the condenser can be removed, as shown in Fig. 31, from which it will be seen that in neither case does any direct light enter the objective, and that an object placed at the point of intersection of the annular beam would be brilliantly illuminated, and therefore seen very distinctly against the black background.

The removal of the front lens gives working distance enough to focus through a considerable depth of water.

Special Condenser.—For microscopes without condenser a lens with ground and blackened centre disc can be obtained, called
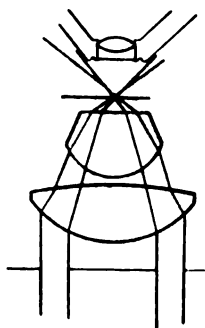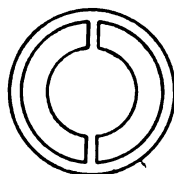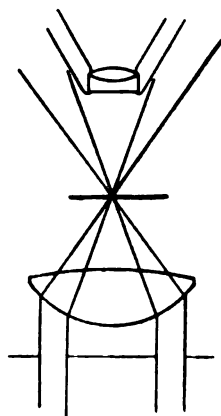
FIG. 29.   FIG. 30.   FIG. 31.

a ' spot lens '; this lens is generally of sufficiently long focus to work through a fair depth of water, but the spot, being fixed, cannot be adjusted to give the best results with any one objective. It will not, therefore, give such good results as a separate stop used in conjunction with an ordinary condenser.

The stops usually sold for the purpose are of metal, as Fig. 30, but better results will be obtained by making one's own of black paper mounted on glass discs, the diameter of the centre being determined accurately for the conditions under which it will be used.

Colour Contrast.—A variation of this method suggested by

Rheinberg consists in making the centre disc, the periphery, or both, of coloured gelatine, the results obtained being both beautiful and instructive.

The above methods suffice for magnifications up to 200 × or rather more, and good results can be obtained with the electric lamp described in the preceding chapter, or even an oil lamp ; but, obviously, the more intense the illumination, within limits, the more brilliant the picture.

HIGH POWER.

For higher powers, owing to the difficulty in centring, focussing, etc., a special condenser is invariably employed, either carried in a centring sub-stage, or fitted with centring screws.
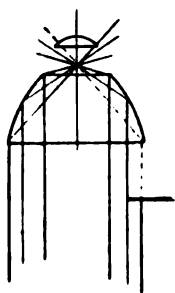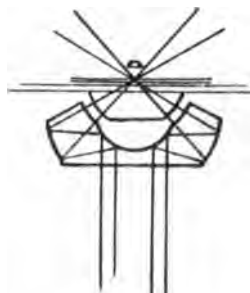
FIG. 32.　　　　FIG. 33.

There are two patterns in general use, the paraboloidal and the concentric, diagrams of which we give above, showing the path of the rays through each.

Both, owing to the extremely wide angle of the rays employed, must be used with an object slip of a definite thickness, with the object to be examined in fluid, and with fluid, water, or oil between the front lens and the under-surface of the object slip.

The aperture of the objective used with either must also be reduced below N.A. 1·0 to cut out any direct rays which might otherwise be transmitted ; this limiting diaphragm, which is dropped on to the back lens of the objective, can be somewhat wider in aperture in the case of the paraboloidal form, although

it should still be under N.A. 1·0, as the condenser is fitted with an iris diaphragm, which acts somewhat as a fine adjustment, the closing of which cuts off any rays which would otherwise enter the objective, as shown by the dotted line in the diagram (Fig. 32).

The range of magnification for which this method is suitable is 500 to 1,000.

The candle-power required for efficient use is 100 to 500.

For further particulars of the use of these high-power dark-ground illuminators see Chapter XII., 'The Microscope in Medicine.'

STANDS

In the preceding chapters we have surveyed in the briefest possible manner the essential parts of a microscope—*i.e.*, the lenses. We now have to consider the best method of mounting these various parts so that there shall be no loss of optical efficiency through mechanical failure.

At first sight this may seem a very simple matter, but it must be remembered that few, if any, other pieces of mechanism have to stand such searching tests; any shake due to looseness of fit is always magnified, sometimes to the extent of 1,000 ×, and yet the various movements must be free enough to allow of adjustment during observation.

By a process of elimination finality has been practically reached as regards the main movements of the present-day instrument, all alike being fitted with a diagonal rack and pinion coarse adjustment, by means of which the object can be brought into view, even with the highest powers and with a lever fine adjustment, which in the smaller stands is usually of a vertical, and in the larger research instruments of a horizontal, type. That this standardization of the essential movements of the microscope has not been attained but by much effort is well shown in a collection of historical instruments such as that in the possession of the Royal Microscopical Society (see Appendix III., p. 86).

Similarly the various types of mechanical movement for the stage have resolved themselves into two: an attachable pattern for the smaller stands and a built-in pattern for the larger instruments.

The stability of the instrument as a whole is assured, in the former by weight rather than spread of base, the adoption of the so-called horse-shoe pattern being now almost universal for

laboratory instruments, which are usually used in the vertical position; while the larger research instruments are more often fitted with a tripod foot, giving maximum stability at any inclination.

All these points will be found illustrated in the next following pages, and in Appendix II., p. 81, particulars of gauges, the universal adoption of which has made possible the interchangeability of apparatus by various makers.

Many attempts have been made to design a stand which shall be universal, but the perusal of these pages, and a careful examination of the illustrations of the types which follow, should prove, even to the novice, that such a consummation is neither possible nor desirable. At the same time, it must be remarked that there has been very little excuse for the numberless patterns which have been produced in the past, apparently merely with the idea of bringing forward something new; such a method is suicidal in these days of machine construction, and it is to be hoped that, in the future, makers will confine their efforts to the production of the types specified by the British Science Guild, particulars of which will be found in Appendix II., p. 82.

It should be remembered, however, that these specifications were drawn up by scientists for professional work, and the naturalist would do well to choose, for his first outfit at any rate, something of lower power, preferably binocular. It is true that this type figures scarcely at all in makers' catalogues at the present day, but it is equally true that the old, simple types of binocular microscope, of which there is always a plentiful supply to be obtained second-hand, are more suitable for elementary natural history than those specified by the British Science Guild.

But for the fact that, being practically indestructible in the hands of the amateur, they can be obtained second-hand in sufficient quantities to meet the demand, never very great, they would doubtless figure more largely in makers' catalogues, which, on account of their omission, are apt to mislead the beginner into thinking such types obsolete and useless, which is very far from the truth.

We will now proceed to illustrate and describe a series of typical stands.
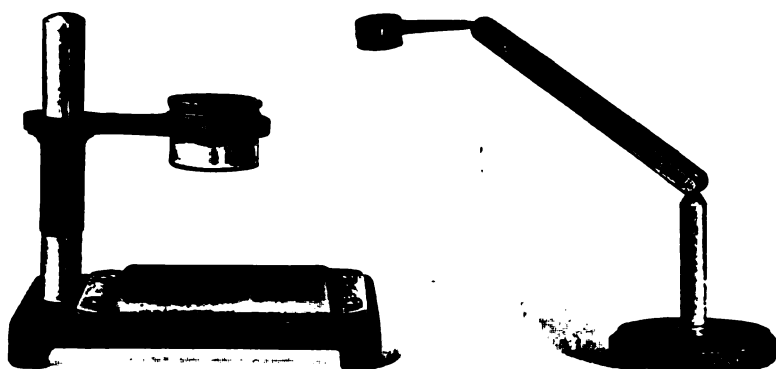
FIG. 34.—HOLDERS FOR LENSES.

The former is focussed by sliding it up and down the rod, the object being laid on the opal glass base-plate ; the latter by bending the jointed arm, which is long enough to reach well over a large object.

FIG. 35.—STAND WITH WRIST RESTS AND RACK AND PINION ADJUSTMENT FOR FOCUSSING LENSES, AS FIG. 5, p. 7.

The stage aperture, which is large enough to hold a small glass dish, is fitted with removable disc of clear glass, and is illuminated by an adjustable mirror.

FIG. 36.—PORRO ERECTOR, THE INTERNAL CONSTRUCTION OF WHICH IS SHOWN IN FIG. 15, p. 12.

It can be carried on a stand similar to Fig. 35, p. 31 ; low-power objectives are screwed into the lower end, the combination giving an erect image.

FIG. 37.—THE STEPHENSON BINOCULAR MICROSCOPE WITH WRIST-RESTS AND RACK AND PINION FOCUSSING ADJUSTMENT.

For diagram of path of rays through this erecting system, see Fig. 14, p. 12.

FIG. 38.—THE GREENOUGH BINOCULAR MICROSCOPE, WHICH CONSISTS OF TWO COMPLETE MICROSCOPES DIRECTED TO THE SAME POINT ON THE OBJECT. THE ERECTING SYSTEM EMPLOYED IS THAT OF PORRO, FIG. 15, p. 12.

It is provided with folding arm-rests, rack and pinion focussing adjustment, inclination-joint, and mirrors.
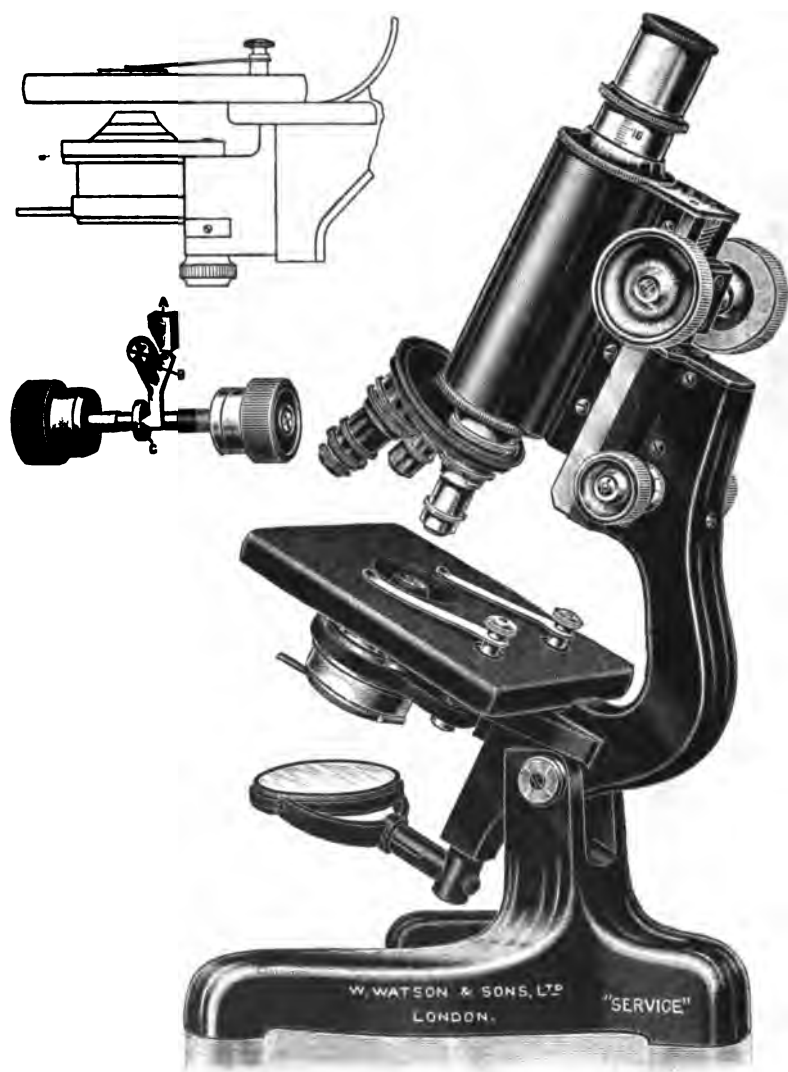
FIG. 39.—LABORATORY MICROSCOPE MADE TO BRITISH SCIENCE GUILD SPECI-
FICATION AND ROYAL MICROSCOPICAL SOCIETY GAUGES THROUGHOUT. SEE
APPENDIX II., p. 81.

The supplementary figures in line show the details of the screw focussing and
swing-out mount of the Abbe condenser, hidden by the tailpiece in the main
illustration, and also the internal mechanism of the vertical lever fine adjustment
for focussing the higher powers.
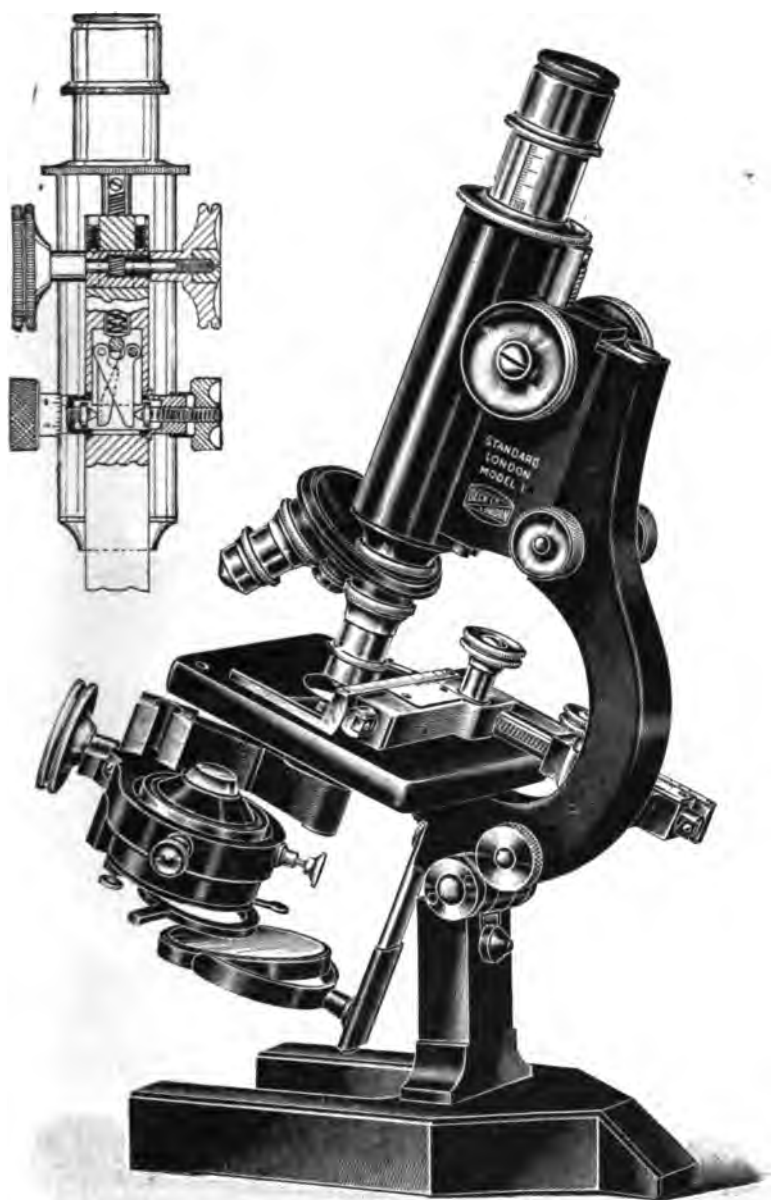
Fig. 40.—Laboratory Microscope with Attachable Mechanical Stage and Rack and Pinion Focussing Swing-out Mount for Abbe Condenser with Centring Screws. (Gauges and Specification as Appendix II.)

In line the mechanism of the two-speed fine adjustment.
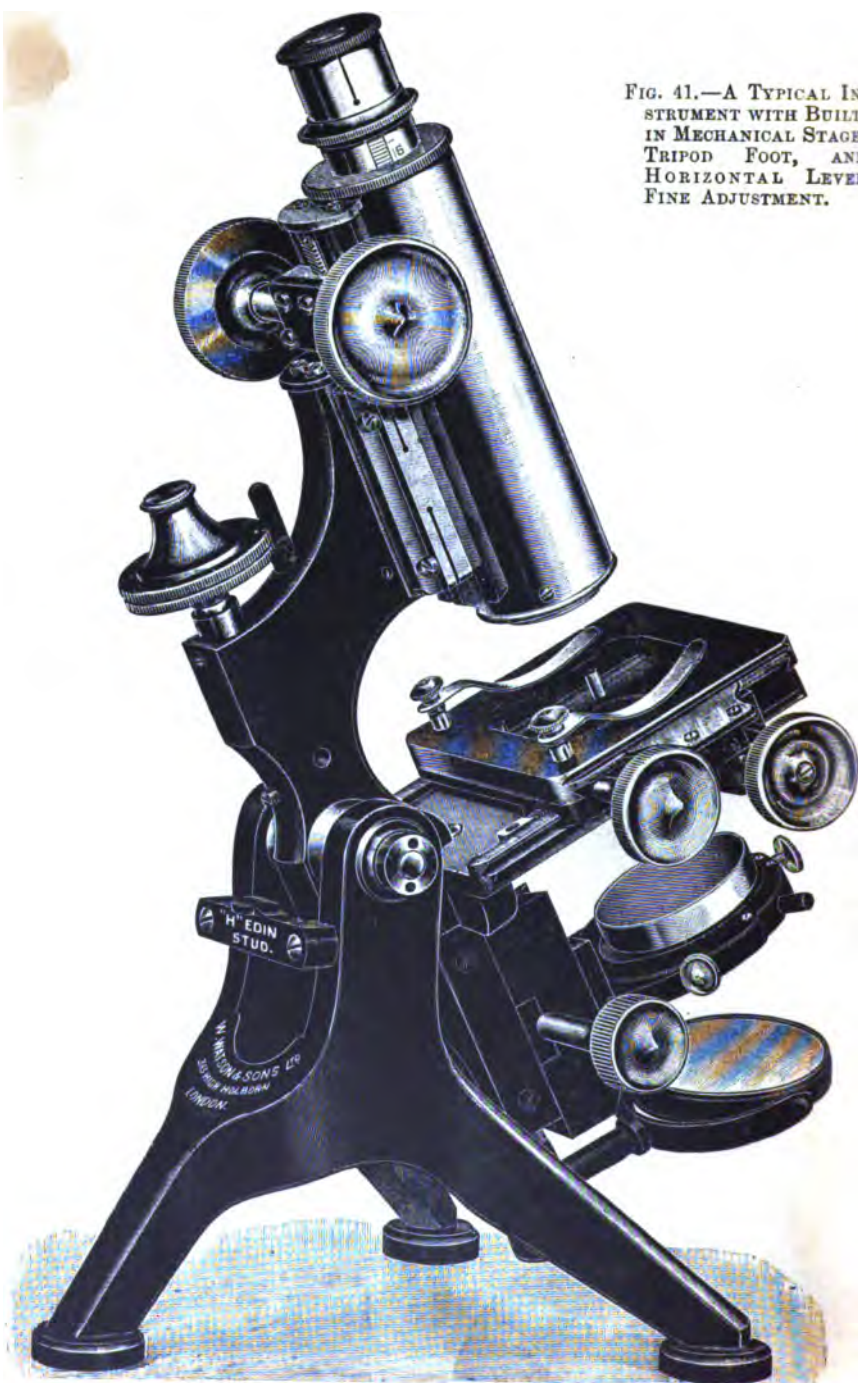
FIG. 42.— AN INSTRUMENT
CAPABLE OF THE HIGHEST
RESEARCH, FITTED WITH
COMPLETE ROTATION TO
STAGE, RACK AND PINION
ADJUSTMENT FOR TUBE-
LENGTH, AND FINE AD-
JUSTMENT TO SUB-STAGE.
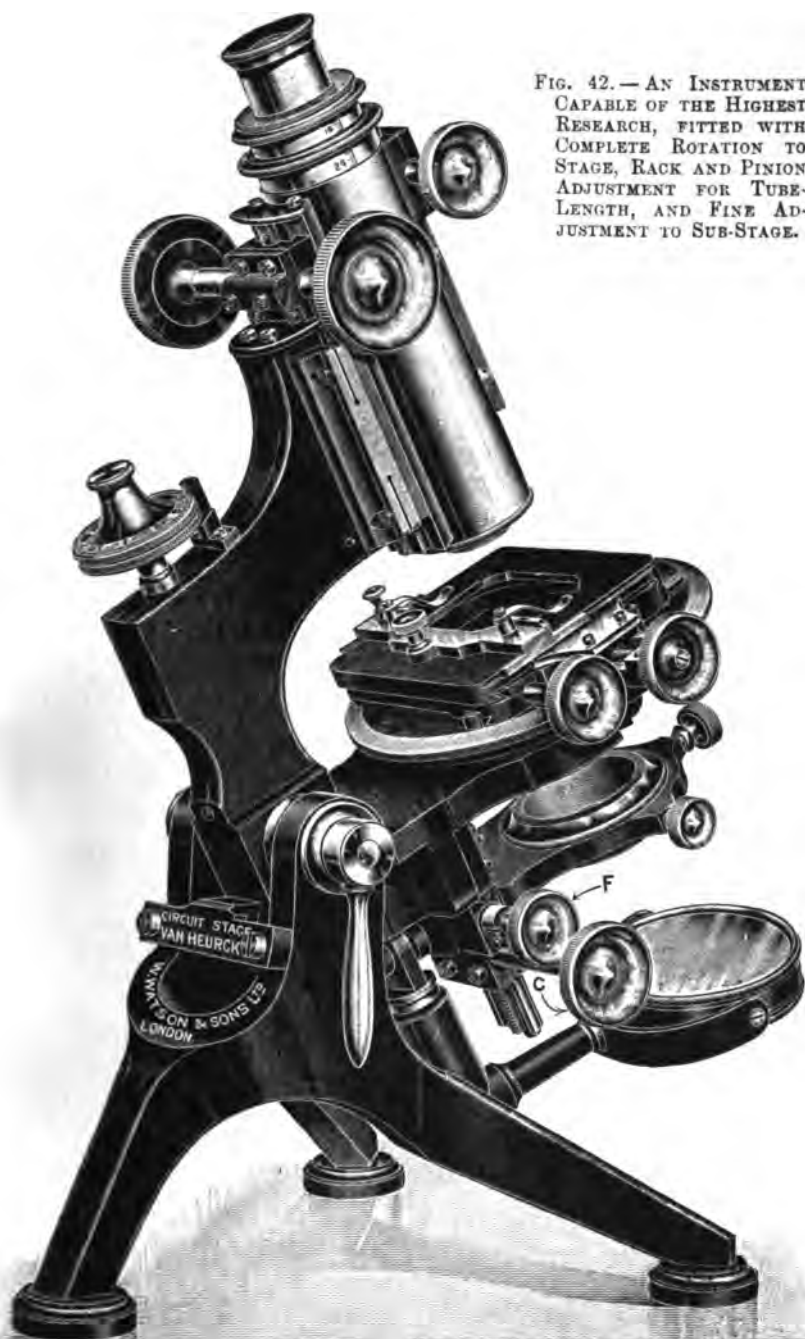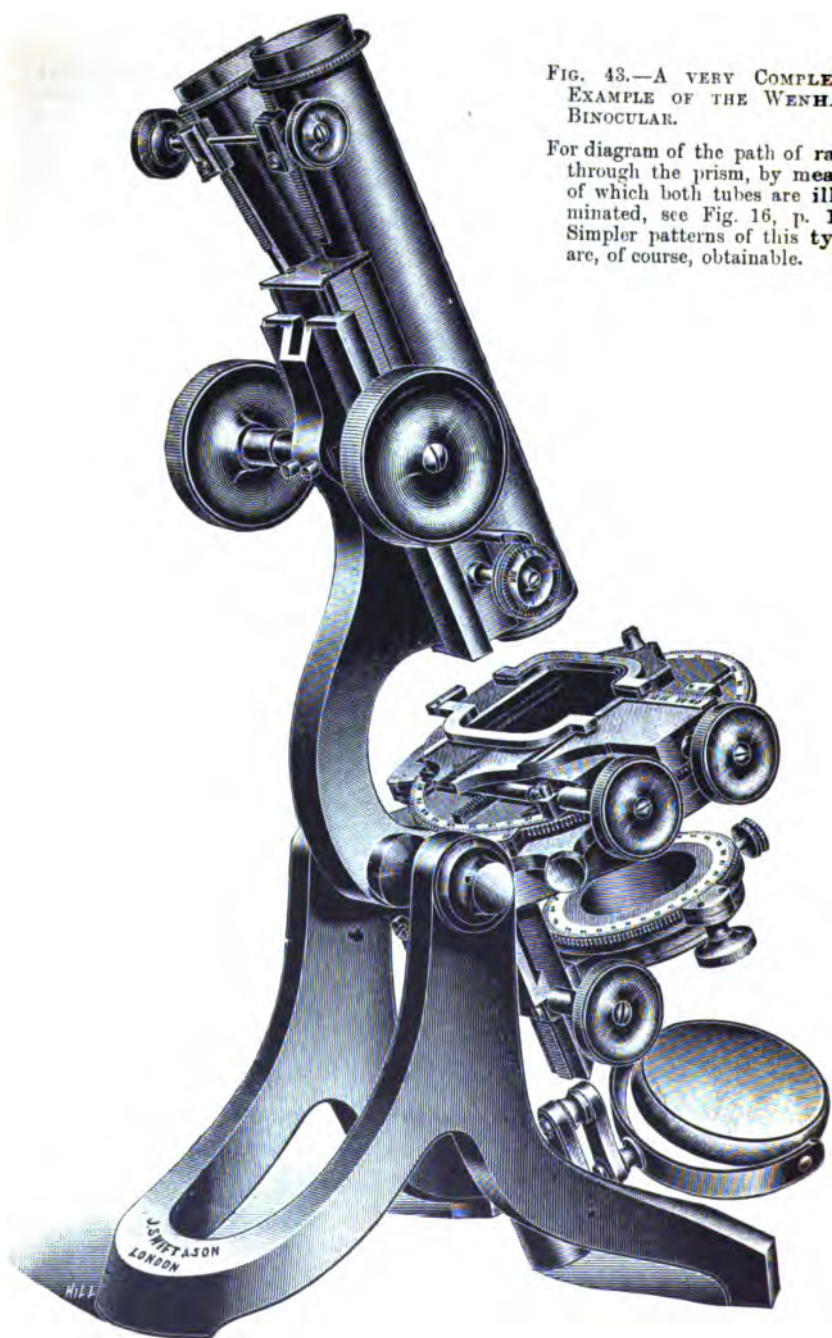
FIG. 43.—A VERY COMPLETE
EXAMPLE OF THE WENHAM
BINOCULAR.

For diagram of the path of rays
through the prism, by means
of which both tubes are illu-
minated, see Fig. 16, p. 14.
Simpler patterns of this type
are, of course, obtainable.

FIG. 44.—THE HIGH-POWER BINOCULAR MICROSCOPE.

The above binocular, unlike the Wenham, can be used with the highest powers without loss of resolving power. For diagram of the path of rays through the prism, by means of which both tubes are equally illuminated, see Fig. 17, p. 14.

ARTIFICIAL ILLUMINANTS

Lamps, Condensers, Screens.

THE advantages arising from the use of an artificial source of illumination have already been referred to in the previous pages.
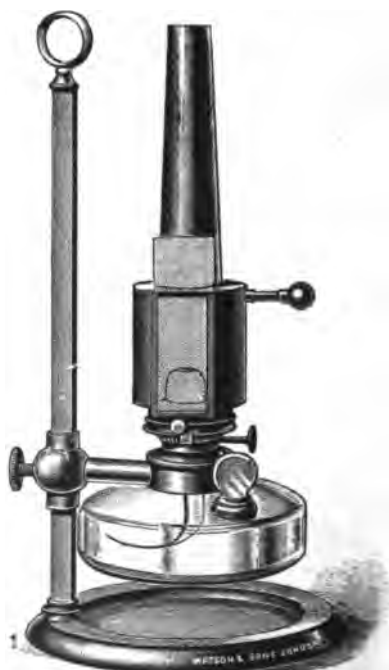
FIG. 45.   FIG. 46.

Of the various patterns available for use with oil, gas, and electricity, the most generally useful are undoubtedly the electric lamp with frosted bulb (Fig. 45) for routine work (see Chapter VIII.), and the oil lamp (Fig. 46) for general use, including research work with the highest powers (see Chapter IX.)

The **Oil Lamp** possesses most, if not all, the good qualities to be looked for in an artificial illuminant : the source, in this case a flame burns steadily without flickering, can be brought to within a few inches of the table, or raised above the stage of the microscope for the illumination of opaque objects ; when set on edge, not broadside on, as illustrated, it can be sharply focussed in the plane of the object, and the divergent light
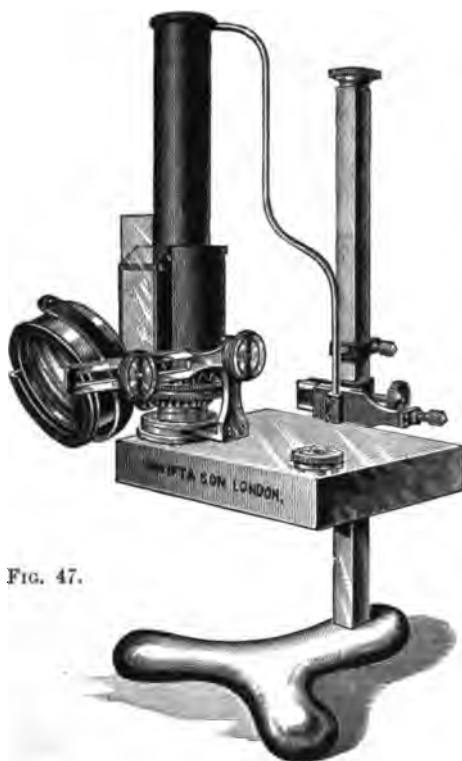
FIG. 47.

emitted can be rendered parallel or convergent by means of a bull's-eye condenser, which can be attached either to the lamp, as Fig. 47, or mounted on a table stand, as Fig. 52A, p. 45.

It is sufficiently brilliant to permit the use of a monochromatic screen, such as Gifford's, but for purer monochromatic light, and dark-ground illumination with the higher powers, a more powerful source must be employed. This want of brilliance for certain work, combined with the messiness of oil, has led to the introduction of various patterns of gas and electric lamps.

Gas Lamps.—Of these there are three types, all incandescent.

Fig. 48.

(1) The inverted gas mantle, as used for domestic lighting, suitably mounted, as Fig. 48, a variation of which adapted for use with spirit is shown in Fig. 49.
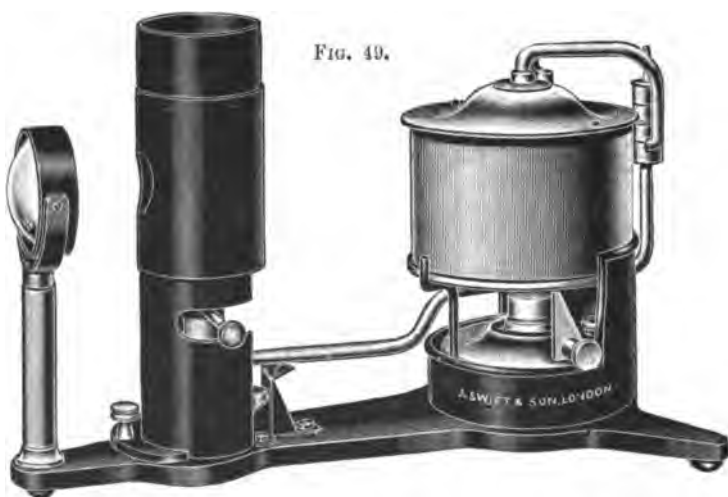
Fig. 49.

(2) The thorium disc lamp, in which a small disc of thorium is raised to incandescence by means of a gas flame impinging on it, of which there are two patterns on the market, the Traviss and the Biss.

(3) The Barnard lamp, in which a metal tube containing material capable of being made incandescent, rolled in the form of a cigarette, is held at right angles to a bunsen flame, in which the protruding end of the cigarette becomes incandescent (Fig. 50).
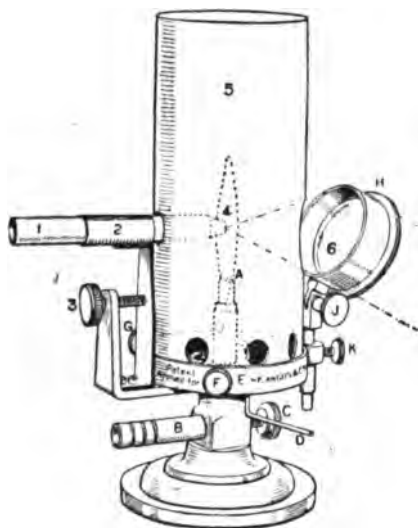
FIG. 50.

These three gas lamps are named in their order of intensity. All are useful for dark-ground illumination, the first and second for low and medium powers, the third for high powers.

The second can, in addition, be used for direct illumination, as the disc can be sharply focussed in the plane of the object, in the same manner as the flame of the oil lamp, without showing any sign of structure.

Electric Lamps.—Of these other than the one already noticed there are also three types:

(1) The Pointolite (Fig. 51), in which a small tungsten ball enclosed in the usual exhausted bulb is rendered incandescent by the current (see pp. 97, 98, and 198).

(2) A miniature form of arc lamp, burning carbons in air, with either hand or clockwork feed.

(3) The mercury vapour arc lamp contained in a quartz tube (see pp. 98, 99).
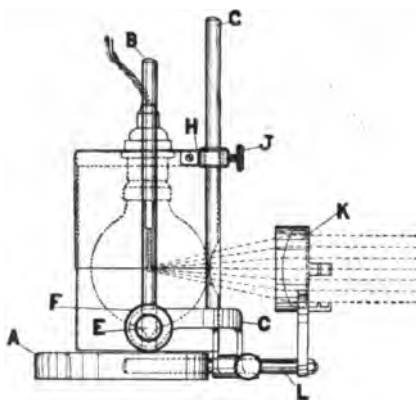
Fig. 51.

The first and second of these are useful for high power dark-ground illumination, and, on account of their great intensity, can be screened to give monochromatic light of greater purity than can be obtained with an oil lamp; but the third is pre-eminently *the* source for monochromatic light, as the incandescent mercury vapour itself emits light of but few wave-lengths within sharply defined limits, which can more easily be dealt with by means of screens than a source giving a continuous spectrum.

Illuminant Condensers.

The efficiency of any form of artificial illumination can, for many purposes, be greatly increased by interposing a condenser, by means of which parallel or convergent light can be obtained from the divergent rays emitted by the illuminant.

This lens is often attached to the lamp (Figs. 47 and 51), and this is undoubtedly the best plan when used only for transmitted light; but it is, perhaps, handier for general use on a separate mount, as Fig. 52A, which shows the ordinary bull's-eye condenser consisting of a single lens, such as is usually employed for illuminating opaque objects.

Fig. 52B shows a two-lens aplanatic condenser with iris diaphragm and centring screws, as mounted for a photomicrographic apparatus or optical bench.

FIG. 52A.   FIG. 52B.

An extremely simple but efficient condenser for transmitted light can be made out of the field lens of an eyepiece, as suggested by Nelson.

Screens.

Monochromatic.—In the preceding pages it has been shown that definition depends largely on the degree of achromatism attained—that is, the correction of the error inherent in a single lens, the outline of the image given by which is surrounded with colour fringes due to the unequal refraction of light rays of

varying wave-length or colour, resulting in unequal magnification
and consequent colour fringes.

If, therefore, the light rays in the illuminant responsible for
the greatest amount of aberration can be cut off before they
reach the microscope, a considerable improvement in definition
will be effected; this can be done by means of light filters, or
coloured screens, so chosen that they absorb the rays it is
desired to eliminate.

Such screens, or filters, can be liquid or solid. One of the
best for general use is the Gifford screen, passing blue-green
rays only, which can be had in either form. More perfect
monochromatic illumination can only be obtained by having
screens specially designed for a particular illuminant, such as
the mercury vapour lamp (see pp. 44 and 98).

Contrast.—A range of Wratten colour screens, the 'M' set,
which can be had in glass or gelatine, will be found almost
indispensable in photomicrography, as by their use the contrast
between adjacent details or between detail and background can
be accentuated.

Intensity.—A pale-blue glass disc, inserted in the stop carrier,
is usually recommended to reduce glare, but, in cases where the
maintenance of colour values is of importance, as in some
medical diagnostic work (see p. 97), discs of semi-platinized or
half-silvered glass will be found more satisfactory. Such
modifiers have the added advantage that the intensity of
illumination can be controlled to a nicety by using a set in
which the film deposited ranges from light to heavy.

METHOD OF USE—ELEMENTARY (FOR ROUTINE WORK)

In this chapter we shall deal with the care of and method of using the simpler models for routine work, by which we mean the determination of the presence or absence of some organism or structure already well known.

By simpler models we have in mind instruments the complexity of which does not exceed that shown in Fig. 39, p. 34, when fitted for routine work with three objectives on a revolving nosepiece, two eyepieces, and an Abbe condenser with iris diaphragm and means of focussing same, the range of magnification being approximately $60\times$, $240\times$, $600\times$, with the low eyepiece, and $100\times$, $400\times$, $1,000\times$, with the high eyepiece.

Care of the Instrument.

The most important point in the care of any instrument of precision is to keep it as free as possible from dust; this can only be done by replacing it in its case when not in use, or by covering it up in some way, by far the most satisfactory cover being a glass shade, a specially thick and correspondingly strong pattern of which can be obtained, called a bell glass, which has a knob at the top, by means of which it can be placed in position and removed.

To Clean Mechanical Parts.—But careful as one may be, however, a certain amount of dust is sure to accumulate. This should be periodically removed by means of a soft brush and a duster before it has had time to work into the moving parts and form, with the oil, a clogging, rather than a lubricating mixture. A drop or two of oil may be required at fairly long intervals on all

bearing parts, but it is useless to add oil if the movements are gummed up with dried immersion oil and balsam, neither of which can be considered a lubricant; should any one of the movements become stiff from this cause, the fitting should be washed out with xylol sparingly applied, and afterwards relubricated.

The stage will also require wiping with xylol from time to time; if bleached thereby, the vulcanite can be restored to its original blackness with a little vaseline well rubbed in.

To Clean Optical Parts.—Dust on the lenses is often a source of greater trouble, because it is not so easily located by the inexperienced, but rotation of upper lens of eyepiece, lower lens of eyepiece, objective, or condenser, keeping one eye on the offending particles, or rather the images of same, will soon prove which component requires attention.

The three surfaces most likely to need wiping are the outer surface of the eye-lens of the eyepiece, the inner surface of the lower or field lens of the eyepiece, and the upper surface of the condenser ; these can be cleaned with a piece of old handkerchief, moistened if necessary with xylol, not the duster used for the metal work, and not chamois leather nor Selvyt, unless it be kept in small pieces in a covered jar, as both are apt to pick up and retain particles of grit.

The front lens of any objective can easily be wiped, and the back lens should not get dusty readily if care be taken to stand it front upwards on the table when removed from the microscope for any purpose, and to see that, when on the microscope, the nosepiece cover is never left in an intermediate position, but clicked home, and that an eyepiece is always in position.

Despite these precautions, should an objective require cleaning, as it will do in the course of years, it is better to return it to the maker than to attempt the job oneself, as, simple as it may seem, it is not an easy job to clean perfectly without scratching a disc of glass a fraction of an inch in diameter at the bottom of a miniature well.

To Clean off Immersion Oil.—This should be wiped off immediately after use by rotating the lens mount against a piece of old handkerchief until only the bead of oil on the lens is left, which is so small that one or two strokes of the wiper across the face of the lens are sufficient to clean it perfectly.

If, through an oversight, oil is allowed to dry on a lens, more should be added, by which means the dried oil becomes liquefied ; the damping of the wiper with xylol is helpful in such cases, as it renders vigorous rubbing unnecessary, the chief fault to be guarded against when cleaning lenses.

Method of Use.

Set up the instrument in front of a window, if daylight is to be used, avoiding direct sunlight; or in front of any artificial illuminant, such as the electric lamp described in Chapter IV., p. 17, and figured as No. 45, p. 40.

1. Set the draw tube at the point for which·the objectives are adjusted, and, bringing the plane mirror uppermost, tilt it until the light is reflected up through the condenser, low-power objective, and eyepiece to the eye, making sure that the diaphragm controlling the amount of light passed by the condenser is wide open and that the objective is held central by the clicking spring on the nosepiece.

2. Focus the low-power objective on the object by means of the rack and pinion adjustment, and the condenser to give maximum illumination; cut down the amount of light by means of the iris diaphragm until the image is free from glare.

3. The medium power may now be rotated into position, which can be done without fear of damaging the specimen, as the low and medium power objectives are made par-focal for the tube-length for which they are adjusted—that is to say, that both focus in approximately the same plane; it is only necessary, therefore, to use the fine adjustment when changing from low to medium power or *vice versa.*

4. It will be found that, with the medium power in position, the diaphragm of the condenser can be opened somewhat, and the draw tube, which for the low power was set at the nominal tube-length, because such powers are insensitive to fine degrees of difference, can now be adjusted with advantage to compensate for any slight difference in thickness between the cover-glass of the object under examination and that for which the objective was set during the course of manufacture.

4

5. If the object requires the highest magnification, the objective $\frac{1}{12}$ inch oil immersion can now be brought into position, first racking up the body tube to avoid the possibility of breaking the specimen, owing to the extremely close working distance of this objective, and also to allow space in which to apply the requisite drop of oil; a very small quantity is sufficient, about the size of a large pin's head. It should be placed on the specimen as near as possible to the optic axis of the instrument, which will be, of course, the centre of the front lens of the condenser.

Into the drop so placed the objective is lowered by means of the rack and pinion, observation being kept on the operation, not by looking down the tube, but by watching at one side, with the eye on a level with the object, until one finds, sight aiding touch, that the front of the objective is touching the cover-glass of the object.

A very slow reverse movement, still using the coarse adjustment, but watching this time for the image through the eyepiece, will, more particularly if the object is a well-defined one, bring it into view without difficulty, when recourse may be had to the fine adjustment, and, if necessary, to the higher power eyepiece.

6. The amount of light passed by the condenser can now be looked to and, if possible, increased, but, unless the object is a very translucent one, it will be found that the medium-power objective utilized all the light that the condenser was capable of passing.

7. Finally, the tube-length can be readjusted, as, although the question of cover-glass thickness is in this case practically eliminated by the use of oil, these high-power objectives are so sensitive to almost imperceptible differences in the conditions under which they are used, and so difficult to adjust to an exact tube-length during the course of manufacture, that a variation of tube-length of $\frac{1}{4}$ to 1 inch either way seldom fails to improve the image.

The procedure would be the same whatever the illuminant used, but if it were an oil lamp, or other illuminant with a small emission area, the whole field would not be lighted when using the low and medium powers ; this is not much disadvantage in the medium power, as the band (image of the flame edge on, in

which position it gives most light) or disc of light is of sufficient area, in comparison with the unlighted portion of the field, as to cause little inconvenience, but with the low power the condenser must be racked down until an area sufficient for observation, or the whole field, is evenly lighted.

METHOD OF USE—ADVANCED (FOR RESEARCH WORK)

In the preceding chapter we described the easiest method of setting up a microscope for routine work, purposely abstaining from any mention of refinements calling for special knowledge or undue expenditure of time, as, no matter what expert microscopists may urge, ninety-nine out of every hundred instruments will always be used thus, and, in our opinion, it is neither desirable nor possible that methods essential for research should be applied to routine work.

It is, of course, true that a microscopist conversant with the full possibilities of the instrument can, given a stand fitted with every possible refinement, optical and mechanical, see all that the routine worker sees with half the power or less, and, employing the same power, can see considerably more; but it is also true that the same apparatus, put into the hands of the routine worker without special knowledge, would show him considerably less than the instruments with adjustments reduced to a minimum, such as are usually used in the laboratories, and would, in many cases, militate against the use of the microscope when it might otherwise be used, on account of the extra time required to set it up.

In our opinion, therefore, the routine worker's outfit cannot be too simple, if it shows efficiently what research methods have already established, even though the efficiency is attained by what, in the hands of the skilled microscopist, is unnecessary power (see Optical Index, Chapter I.), and the research worker's outfit cannot be too elaborate, provided the knowledge of how to use it keeps pace with the elaboration of apparatus, and no apparatus be added unless it tends to increased seeing power, even though it be to the extent of but a fraction of 1 per cent.

We will, therefore, consider the various refinements that have been found essential to maximum resolution.

Essentials.

Lenses of High Quality.—It is, perhaps, hardly necessary to state that the primary condition is the use of the finest optical parts procurable, especially objectives, and the finest objectives are, undoubtedly, the so-called apochromatics originated by Abbe, who first succeeded, by the aid of new optical glasses and fluorite, in practically removing the chromatic aberration of the image due to the secondary spectrum, and in correcting the spherical aberration for all colours.

Objectives of this type are certainly the finest, when judged by white light—that is, light from a source emitting all wavelengths—but the improvement in the correction of the achromatic objectives has been so great of late years that, when similar powers of these two series are compared by monochromatic light, it is very difficult indeed to detect any difference in the images, and, as the cost of the apochromatic objectives is something like three times that of the achromats, there is something to be said for the cheaper series even for research work.

An incorrigible colour defect, of the nature of under-correction (see Chromatic Under-Correction, Appendix I., p. 78), inherent in all high-power objectives, whether apochromatic or achromatic, led Abbe to introduce the same defect, to the same amount, in all the powers of the apochromatic series, in order that it might be corrected by an equal error of opposite sign in the eyepiece. Such eyepieces are called compensating (Fig. 11, p. 9).

This error, although present in the high-power achromats, has not been introduced throughout the whole series. Consequently, while the performance of an achromatic objective $\frac{1}{12}$ inch is improved by the use of a compensating eyepiece, the performance of the other powers is deteriorated, which fact led to the introduction of an adjustable eyepiece, in which the amount of over or under correction is variable according to the position of the eye-lens (Fig. 12, p. 9).

Elsewhere it has been stated (p. 22) that the Abbe condenser is limited in efficiency to an objective aperture of N.A. 0·70;

it is evident, therefore, that for research work an aplanatic, or even an oil immersion condenser, should be used; the former will suffice up to an objective aperture of N.A. 1·30, but for the highest obtainable objective aperture, N.A. 1·40, the latter mounted in sub-stage with fine adjustment as Fig. 42, p. 37.

Adequate Illumination.—A small source which can be focussed in the plane of the object is essential, and, for all work for which an approximation to monochromatic light is sufficient, there is nothing better than an oil lamp, as figured and described in Chapter VII., with a blue-green light filter and the flame set edge on ; but for monochromatic light of greater purity, either the mercury vapour arc or Pointolite lamp, suitably screened, will be found more efficient, owing to the greater intensity of illumination.

Full Utilization of Effective Objective Aperture.—There exists an intimate relationship between the power and N.A. of the condenser and of the objective.

The power should be such that the image of the flame forms a broad band set vertically in the field of view, the space on either side being comparatively dark.

The N.A. should be sufficient to fill three-quarters of the back lens of the objective with a solid cone of light ; the possibility of doing this can be determined by focussing the objective on an object mounted in balsam, and the condenser, with diaphragm fully open, also on the object, so that the image of the lamp flame is seen superimposed on that of the object, which should then be moved slightly so that the rays of light pass through a clear film of balsam only ; on removing the eyepiece, the back lens of the objective will be found filled, or nearly filled, with light, according to the respective apertures of condenser and objective.

Owing to the depth from back to front of the lamp flame, there is some little latitude in the position of the condenser ; it can usually be racked up slightly until a point is reached at which the solid cone, or homogeneous illumination of the back lens, begins to break up, as in diagram, where an attempt to increase the area of homogeneous illumination shown in Fig. 53 as too small to fill the middle ring, representing the diaphragm aperture set for ¾-cone, results in an appearance as Fig. 54,

proving that the N.A. of the condenser is too small, the appearance required being as Fig. 55.

This relationship of power and N.A. of condenser with the power and N.A. of objective would seem to imply the necessity for a range of condensers, but in practice it will be found that the more sensitive objectives, $\frac{1}{6}$ to $\frac{1}{12}$ inch of N.A. 1·30, can be served with an aplanatic condenser of N.A. 1·0, as the removal of the front lens reduces its power to that most suitable for an objective $\frac{1}{6}$ or $\frac{1}{8}$ inch without reducing the aperture to a point below that required by such objectives. There is not the same need of accuracy with the low powers, and for these the rays of light from the lamp can be parallelized by a bull's-eye, thus illuminating the whole of the· field, and the aperture cut down by the iris diaphragm.
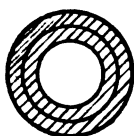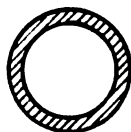
FIG. 53.   FIG. 54.   FIG. 55.

Perfect Centration.—There must, of course, be perfect centration and alignment of the various component parts of the optical system, hence the necessity of using a stand with rack and pinion focussing and centring sub-stage, as Fig. 41, p. 36, with fine adjustment, if an oil immersion condenser be used.

Test of Adjustment.—Seeing that in research work the object is more or less an unknown quantity, it is essential that the adjustment of the instrument should be checked on setting up, and from time to time during use, by reference to test objects, as they are called, the structure of which is known.

These should be, preferably, objects showing definite periodic structure, such as the silicious skeletons of certain of the Diatomaceæ, of which the most useful in this connection is *Pleurosigma angulatum*, which shows under the higher powers, after removal of the eyepiece, a characteristic diffraction, or back lens image, consisting of six primary spectra arranged as a hexagonal pattern about the central beam.

Mechanical Draw Tube.—Owing to variable thickness of

cover-glass, often unknown and unknowable once used, means should be provided, either on the objective mount itself, by correction collar, which is unusual nowadays, or on the stand, for compensating for the variation between the thickness of the cover-glass with which the object under examination is covered and the thickness of cover-glass for which the objective was corrected in the course of manufacture.

This same adjustment will serve also for accurately determining the tube-length for which the oil immersion objective has been corrected, which differs somewhat in nearly every case from the nominal tube-length.

The sliding draw tube is, of course, sufficient for rough adjustment, as described in Chapter VIII., but to obtain the utmost accuracy it is necessary to observe the image whilst making the adjustment, and this can only be done by having a rack and pinion adjustment fitted to the draw tube, as in Fig. 42, p. 37. For further note on this correction see Chapter XI.

Method of Use.

It is only necessary to draw together the various points referred to above by detailing the steps necessary to set up a research microscope fitted with three objectives—low, medium, and high—adjustable eyepieces, and aplanatic sub-stage condenser mounted on a stand, as Fig. 42, p. 37, taking as an object the diatom referred to above, *Pleurosigma angulatum*, and as illuminant an oil lamp with parallelizer (Fig. 47).

1. Set microscope and lamp in alignment with the flame of the lamp edge on ; if the nature of the object will allow, as in this case, the stand can be inclined to a convenient angle and the mirror dispensed with, otherwise the plane one should be used.

2. With low-power eyepiece and objective in position, tube-length adjusted to that for which the objectives are nominally corrected, and the object, *Pleurosigma angulatum*, on stage, focus both objective and condenser.

3. Close the diaphragm of the sub-stage condenser to a pin-point, and rack back the objective until the image of the iris diaphragm is seen.

4. Adjust centring screws of sub-stage, if necessary, to bring this aperture central.

5. Rack objective down again to focus of object, and, if necessary, alter the position of the lamp until the flame bisects the field vertically. It should be approximately 10 inches from mirror.

6. Remove front lens of condenser, set up parallelizer in front of lamp in order to fully illuminate the field of the low power, focus the sub-stage to give maximum illumination, reduce aperture of condenser to ¾-cone,* and insert coloured screens if the illumination is still too brilliant for the eye; finally, adjust eyepiece and iris diaphragm of condenser.

7. Proceed to examination with the medium power by removing parallelizer, after which refocus condenser, bring lamp flame central by altering the lateral screw of sub-stage if, owing to some slight difference in the centres of the objectives, such adjustment be necessary, remove eyepiece, and adjust the diaphragm of condenser to ⅔-cone; replace eyepiece, adjust tube-length for cover-glass thickness; finally, adjust eyepiece and the iris diaphragm of condenser.

8. To examine with the high-power objective $\frac{1}{12}$ inch oil immersion, with the medium-power objective still in position, replace front lens on condenser and refocus, adjusting lateral centring screw if necessary, rack up body tube, and rotate objective $\frac{1}{12}$ inch into position; apply oil to object, and rack down, watching the movement from one side, with the eye level with the object, until the cover-glass is lightly touched; then, watching for the image, rack up slowly until it appears, adjust lateral screw of sub-stage if necessary, and amount of light passing to the objective by removing eyepiece and shutting down the diaphragm to ¾-cone, replace eyepiece, adjust tube-length, eyepiece, and iris diaphragm of condenser.

* This ⅔-cone illumination, advocated by Nelson as giving maximum resolution, is intended merely as a guide. The final adjustment of the iris diaphragm of the condenser is dependent on the nature of the specimen and the quality of the objective.

RECORDING APPARATUS

Position (Finders), Size (Micrometers), Shape (Drawing Apparatus), Detail (Photomicrographic Apparatus).

THE methods used for recording observations range from the very simplest, calling for nothing more than an engraved mark on the stage of the microscope, to the most complex apparatus such as is used in high-power photomicrography.

Position (Finders).

It is obvious that, when dealing with an object invisible to the unaided eye, the first step towards a record of any sort is to devise some means by which one may be able to find it again readily whenever required.

Single Mark.—The simplest means of doing so on a plain stage is to engrave a single dot, which can be filled in with white paint, about the size of a very small pin's head, on the surface of the stage, at such a distance from the optic axis of the instrument, either right or left, that it will not be hidden by the label when any part of the specimen is brought into view.

An ink or diamond mark on the glass over this spot renders it possible to replace the specimen on the stage at any time in approximately the same position, if care be taken to see that, before marking, the long edge of the glass slip is parallel to the front edge of the stage, and that parallax be minimized as much as possible by placing the eye immediately over the spot whilst marking.

Wright's Finder.—With some microscopes, however, having a large stage opening, the spot would be in such a position that it would be frequently hidden by the ordinary label, as used on a 3 × 1 inch slip, and, as the method adopted must be

applicable to any specimen, a series of squares, called a Wright's finder, is frequently engraved on the right-hand front corner of the stage, by means of which the position of one corner of the slip can be noted, the series of lines forming the squares being numbered in both the vertical and horizontal directions for this purpose.

Graduated Mechanical Stage.—When the instrument is so fitted similar references will suffice, but all such marks or references are, of course, only of use for refinding on the same instrument.

Marker.—An attachment is sometimes employed which screws on in place of the objective, and draws on the cover-glass with a diamond a minute circle round the desired field; this is the most direct method, and practically the only satisfactory one, when a particular object or field is to be examined on an instrument other than that on which it was first found.

Size (Micrometers).

Most microscopic objects can only be measured optically, but low-power objects can be measured mechanically.

The Mechanical Method calls for a fixed point or line in the eyepiece, such as a spider's web or hair stretched across the diaphragm or a glass disc ruled with a diamond line placed upon it and a graduated mechanical stage reading by verniers, the readings of the stage being taken when either end of the object it is desired to measure are coincident with the line in the eyepiece, the difference between the two readings being the measurement required; such a method is speedy and fairly accurate for low powers, but optical methods are to be preferred, and for high powers are essential.

The Optical Method.—Measurements can be obtained by:

1. Actual projection.
2. Successive superposition of image of object and scale.
3. Simultaneous superposition of image of object and scale.

The last, owing to the ease and speed with which measurements can be made, is usually adopted; care, however, is required in checking the results, as this method is open to a source of error non-existent in the more cumbersome methods.

All optical methods depend on the use of a stage micrometer, which is merely a finely divided scale ruled with a diamond point to 0·1 and 0·01 millimetre, or 0·01 and 0·001 inch ; on the usual 3 × 1 inch slip.

Actual Projection.—The microscope is set up horizontal as for photomicrography, as described later in this chapter, the image received on a screen placed at any convenient distance, and the two points, the exact measurement of the distance between which is required, taken off by means of a pair of dividers ; the stage micrometer is then substituted for the object and the dividers applied to the image of scale, from which a direct reading is thus obtained.

Successive superposition of image and scale.—This is a similar method to the above, except that a camera lucida is used (for description of which see below) giving a subjective image instead of the objective image given by actual projection; it is not, perhaps, quite so easy to use until one has mastered the use of a camera lucida, but, once this difficulty is overcome, it is quicker and likely to be more accurate, in that the paper on which the images are seen is in a more convenient position for accurate measurement.

Simultaneous superposition of image and scale.—This is the most usual method. The scale referred to is not the stage micrometer, as it is impossible to focus two objects at once in the microscope, but a supplementary scale placed upon the diaphragm of the eyepiece, and known as the eyepiece micrometer, the equidistant spaces of which are calibrated once for all, by means of a stage micrometer, for each objective eyepiece and tube-length used by focussing the stage micrometer upon the stage and adjusting the draw tube until a whole number of divisions of the upper scale exactly superpose a whole number of divisions of the lower scale, from which, the value of the divisions of the lower scale being known, it is easy to find the value of a single division of the upper or eyepiece scale ; but it is essential that the tube-length at which this determination is made should always be used when measuring objects with it, hence the need of careful checking referred to above.

Such a scale in the eyepiece, if the microscope be fitted with a mechanical stage, will be found easy to use, but without some

mechanical means of placing the edge of the object to be measured against one of the lines of the scale the task, especially under a high power, is extremely difficult; a film of water between the object and stage will be found useful when measuring on a microscope without a mechanical stage, as it slows down and regulates the movement of the object slip.

Various sliding and screw micrometer eyepieces have been devised from time to time, but are now rarely used; it is, however, a convenience to have a special eyepiece for the work with adjustable eye-lens, so that the scale can be accurately focussed.

Care should be taken, when calibrating the eyepiece scale, to take as many as possible of the central divisions into account, as the subsequent division by the number taken, in order to obtain the value of each division, tends to minimize any possible error; but the divisions at the edge of the field should not be taken, either when calibrating or actually measuring, as, owing to spherical aberration, they are less accurate than the central ones.

Shape (Drawing Apparatus).

The general outline of objects can readily be sketched by anyone with sufficient accuracy to constitute a record useful for reference or even illustration, if one or other of the various appliances designed for the purpose be used to aid eye and hand, but it is given to few to be able to produce a drawing which shall be both accurate and finished in the artistic sense.

The aids referred to can, as in the case of measurement, be either mechanical or optical.

The Mechanical Method.—All that is required for this method is a glass disc ruled in squares, which is placed on the diaphragm of the eyepiece so that the object is seen ruled, as it were, with a series of guide lines, drawing-paper ruled in squares to correspond, and plenty of patience.

The Optical Method depends on the superimposition of the images of object and paper so that a tracing can be made; this can be done by projection or reflection.

Projection of the image on the paper is by far the easier method, but a very powerful illuminant is required except for

low powers; special inverted microscopes have been made fitted
with an electric arc lamp for such work, but the ordinary micro-
scope can be used if set up with the body tube horizontal and a
reflector be fitted at the eye end to throw the image down on the
paper. This reflector, in order to eliminate the double image
given by the two surfaces, front and back, of an ordinary mirror,
is usually a right-angled prism, as shown below (Fig. 56).
The image thus projected is, however, partially inverted—that is
to say, it is reversed as regards top and bottom, but not side
to side.

Reflection.—If instead of projecting downwards we reflect
upwards by means of a transparent reflector, view the microscope
image by reflection, and look down through the reflector at the
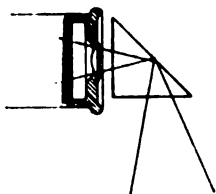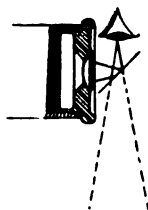
FIG. 56.   FIG. 57.

paper (Fig. 57), making the reflector of neutral tinted glass in
order to eliminate the reflection from the under surface, we have—

The Beale Camera Lucida, which is independent of a powerful
illuminant; in fact, for any but the highest powers we shall have
to reduce the illumination in the microscope until the luminosity
of the image is equal to that of the drawing-paper. This is a
most important point in all subjective drawing methods, ignor-
ance of which accounts for practically all the difficulties experi-
enced in using such apparatus.

Another point which is sometimes overlooked with this type
of reflector is that the distance from eye-point to drawing-paper
must be at least 10 inches, the minimum distance for distinct
vision, which means that the average microscope must be built
up somewhat, and that, if spectacles are worn for reading, they
must be used even though they are not usually used for ordinary
observation with the microscope.

The disadvantage of having to use the microscope in the

horizontal position with the stage vertical, an impossible one with many more or less fluid objects, combined with the partial inversion, a fault which it shares with the projection method described above, led to a modified pattern, viz.—

The Ashe Camera Lucida, in which a double reflection corrects both faults. It is not so easy to use, however, as the Beale, and the position of the microscope, although more convenient, is confined within narrow limits of inclination.

If, therefore, for any reason the Beale reflector is found wanting, the pattern usually chosen is that due to Abbe.
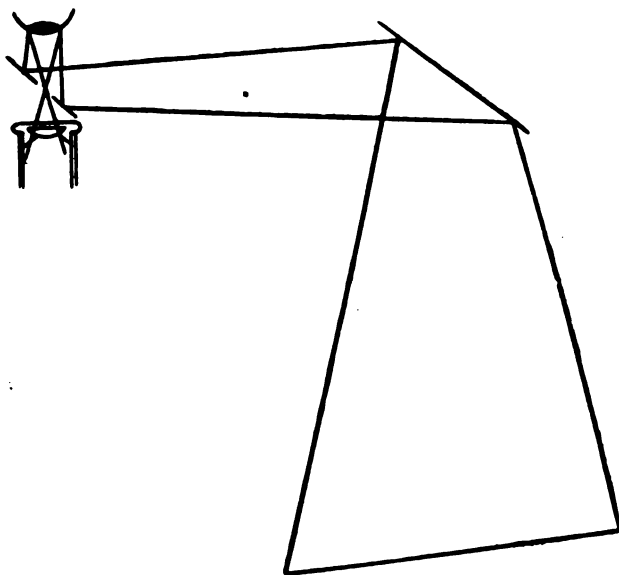
Fig. 58.

The Abbe Camera Lucida.—In this pattern the observer looks directly down the microscope tube, the image of the paper and pencil being transmitted to the eye by means of two reflectors, the smaller of which is mounted directly over the eyepiece, a small aperture in the reflecting surface allowing the rays from the microscope to pass to the eye (Fig. 58).

It is usable with microscope vertical or at any inclination so long as the drawing-board is made to conform to the angle of the stage from back to front; it must also be inclined to the

stage sideways, as shown in the figure, to prevent lateral distortion; the amount of this inclination can be accurately determined by using a stage micrometer as object, the board being inclined until the spaces between the lines are equalized.

This pattern is free from any partial inversion of image, the drawing being an exact copy of the object as seen in the microscope, and if a medium-power eyepiece be used, which should be the case with all the patterns described (unless adjustment is provided for eyepieces with extra long or extra short eye-point), and attention be paid to equalizing the illumination of object and paper, little difficulty will be experienced in using it.

That considerable difficulty is often experienced in using such apparatus is proved by the number of different patterns that have been suggested from time to time, many of which are still obtainable; they all depend, however, on the principles described above, and will present no difficulty to anyone who has grasped them.

Detail (Photomicrographic Apparatus).

We have seen above that it is possible for anyone to make satisfactory drawings of the shape or outline of an object; these outlines can be filled in with a varying amount of detail, according to the ability of the draughtsman to translate what he sees into line, or masses of light and shade. Some objects lend themselves to this method of delineation; others, again, are almost hopeless, more particularly when the detail is of a controversial nature, and its reproduction by hand must needs be open to the charge from one side or the other of being biassed.

It is in such cases that photography will be found most useful, and, the necessary apparatus once installed, it will not be long before the more tedious process of drawing will be ousted, even for those objects which, before its advent, were adequately recorded with the pencil. A number of works have been written on the art of photographing the microscopical image; we do not intend, therefore, to treat the matter exhaustively, but rather to set down as concisely as possible the

necessary apparatus required and the elementary principles which must be understood before attempting such a method of recording observations.

The Apparatus Required.

1. **The Optical Equipment.**—Nothing special is required except it be a projection eyepiece with an adjustable front lens, by means of which the diaphragm, which is smaller than in the ordinary pattern, can be focussed sharply on the screen; but such an addition is not essential, the low-power eyepieces already described giving good results, and a large number of such photographs being taken without any eyepiece whatever.

The sub-stage condenser becomes perhaps of rather more importance than in ordinary visual work, the extra light given by the better corrected condensers considerably reducing the time of exposure, a very important detail when the image is so much magnified, the slightest tremor at such magnifications being fatal to definition.

2. **The Stand.**—Any monocular instrument mentioned in these pages, or of similar type, is usable; but a centring sub-stage is almost essential for first-class work, and all internal reflections from the body tube must be eliminated either by inserting a diaphragm or a velvet lining.

3. **The Camera.**—This can be either vertical or horizontal, many varieties of which will be found figured in the makers' catalogues. In either pattern provision should be made for the visual adjustment of the microscope, and the subsequent linking up of the light-tight connection, without the possibility of greater disturbance than can be rectified by the fine adjustment of the microscope. If a long horizontal camera be used, some provision must be made for gearing up the fine adjustment, so that it can be operated while observing the image on the ground glass screen, which can best be done, finally, with a magnifier fixed in a tube so as to focus at the plane occupied by the sensitized surface of the plate, the ground glass screen being exchanged for a clear glass one, which acts merely as a support for the lens.

4. **The Illuminant.**—This depends entirely upon the magni-

fication required; for low-power work an oil lamp with an efficient condenser, such as the Nelson doublet (Fig. 52B, p. 45), will give good results, but the greater the intensity, the shorter the exposure, the importance of which need not again be referred to, and the greater the possibility of obtaining contrast, without unduly lengthening exposure, by the use of suitable coloured screens, a range of which should be in the possession of anyone making use of this method of recording observations.

Method of Use.

The essential point is to get every piece of apparatus into alignment. Supposing, therefore, that the microscope in use has centring screws to the sub-stage, that a horizontal camera is available, and that the illuminant condenser employed to parallelize the rays has an iris diaphragm and centring screws, the following procedure will be found expeditious and reliable :

1. Set each piece of apparatus as nearly in line as possible by measurement, and mark the position of each, adjusting the height of illuminant and illuminant condenser to the optic axis of the microscope.

2. Remove the microscope and set up as for visual observation, replacing it on the base-board when in perfect adjustment, after sliding back the camera front some 12 inches.

3. Test the position of the illuminant condenser in relation to the illuminant by means of a piece of card, to make sure that the illuminant is situated at the principal focus of the illuminant condenser, and that, in consequence, a beam of parallel light is being projected (see bottom diagram, Fig. 18, p. 18).

4. Having determined this distance, adjust the position of the illuminant and illuminant condenser, both vertically and laterally, until this beam of parallel light is projected centrally upon the aperture of the diaphragm of the sub-stage condenser ; this can best be proved by means of the white card.

5. The card should then be set up against the camera front, and an image of the edge of the iris diaphragm of the illuminant condenser, closed down to a very small aperture for the purpose, should be projected through the microscope upon it ; this can be done by racking the sub-stage condenser very slightly in or out of the position at which it was set when placed on the base-board.

If not central with the field, adjust centring screws of the illuminant condenser until centration is attained.

6. Reopen iris of illuminant condenser until the field projected on the card is filled with light, and no more ; adjust projection eyepiece if in use.

7. Check centring of illuminant with illuminant condenser by placing card screen against the sub-stage condenser iris, and adjust, if necessary, by moving illuminant.

8. Having thus obtained an evenly lighted field, bring forward camera front, so as to link up the light-tight connection, and proceed to adjust image on screen, slipping into position any contrast screen decided upon before final focussing.

9. No exposing shutter is necessary, as the sensitized plate can be protected until the moment of exposure by interposing a piece of card between illuminant and microscope.

By carrying out the above instructions, anyone with a knowledge of photography would be able to do good work from the beginning, even with improvised apparatus ; but photomicrography is essentially an art in which, if there is any considerable amount of work to be done, it is cheaper to obtain apparatus the efficiency of which has been proved than to muddle on with makeshifts.

TESTS

UNDER the heading of tests we will now describe the apparatus and methods employed for testing the qualities of an instrument, both optical and mechanical, for which the makers are responsible, and the working adjustments of the instrument, for which the operator is responsible, recapitulating and amplifying where necessary explanations already given, and adding methods which could not be conveniently dealt with in the preceding chapters without loss of continuity.

The tests of quality comprise:

Magnification of objectives and eyepieces,
Numerical aperture of objectives and condensers,
Optical performance of objectives—(a) spherical aberration,
    (b) achromatism,
Mechanical movements,

the findings of which are unalterable by the user.

The tests of adjustment comprise:

Efficiency of illumination, axial and dark-ground,
· Cover-glass correction,
Test objects,
The exact determination of the character of the light
    employed,

the findings of which serve as a guide to possible improvement.

We will proceed to take these various tests in the order in which they are set down above.

Magnification of Objectives and Eyepieces.

Objectives.—The initial magnification can be determined by projecting through it an image of a stage micrometer upon a screen 10 inches from the back lens of the objective.

This method, although not strictly accurate (see below), will suffice for all practical purposes.

The combined magnification of objective and eyepiece can be determined in the same manner, the distance being measured from the point at which the rays cross on emergence from the eyepiece, the so-called Ramsden disc, which can easily be located by holding a small screen of ground glass or card immediately in front of the eyepiece and moving it away until a point is reached at which the disc of light is brightest and of least diameter.

Eyepieces.—From the figure found for the magnification of objective and eyepiece the magnification of the eyepiece itself can be determined, but it must be remembered that the initial magnification of the objective is affected by the tube-length.

For instance, if an objective the initial power of which has been found to be $10 \times$ gives, with an unknown eyepiece on an 8-inch tube, a total magnification of $80 \times$, the power of the eyepiece will be $10 \times$, not $8 \times$, as, in order to arrive at the magnification of the eyepiece itself, we must divide the total magnification by the proportionate part of the initial magnification of the objective developed on an 8-inch tube—viz., $\frac{8}{10}$; then,

$$80 \div (10 \times \tfrac{8}{10}) = 10.$$

The power thus found would differ slightly with the higher, and still more with the lower, powers, owing to the fact that we have taken for our basis in the measurement of the initial magnification of the objective a mechanical tube-length of 10 inches rather than an optical tube-length; but although such points must be taken into account in advanced work, they have no practical bearing on the use of the instrument, the elementary rules for which must be based on approximations.

Numerical Aperture (N.A.) of Objectives and Condensers.

The numerical aperture of an objective can be measured in numerous ways.

Objectives—dry.—No particular apparatus is necessary if some plan such as that suggested by Conrady be adopted.

The procedure is as follows:

On a dark background, such as a table, place two white cards

with their inner edges parallel to each other and a suitable distance apart. If a dry objective is held at a sufficient distance above the table and directed towards a point midway between the two cards, images of the latter will be seen at the back of the objective, and by approaching the objective to the table these images will recede from one another, until finally they can be got to disappear under the margin of the back lens.

It is obvious that when this disappearance takes place the inner edges of the two cards are lying in the direction of the most oblique rays which can enter the objective. In other words, they form, with the focal point of the objective, its angle of aperture.

In order to get numerical results, the two cards must be placed at a measured distance apart, and the objective made to slide up and down along the edge of a divided scale, such as an ordinary foot rule.

When the point of disappearance of the images of the inner edges of the cards has been reached, the distance from the front of the objective to the table is read off on the scale. This must be corrected by deducting the working distance of the objective —that is, distance between objective-front and object when used on the microscope in the ordinary way. Then :

1. If a table of trigonometrical functions is available, divide the corrected distance from objective to table by half the distance between the inner edges of the two white cards. The quotient is the cotangent of half the angle of aperture ; find its value in the table, and take from the table the sine of the same angle. This is the desired numerical aperture.

2. If no trigonometrical table is available, the numerical aperture is found as follows :

Square the reduced distance from objective to table, also half the distance between the two cards. Add the two squares together and extract the square root of the result. Then the numerical aperture is found as the quotient of half the distance between the pieces of card and the value of the above square root.

In order to make the calculation involved as simple as possible, it is manifestly an advantage to make one of the lengths entering into the calculation unity, which is easily done by placing the two cards a distance of two units apart.

For objectives of low numerical aperture 2 inches will be found a suitable distance ; for those of higher numerical aperture a distance of 2 decimetres. This last measurement is suggested so that the experiment may be in its simplest form.

Objectives—immersion.—For these a special piece of apparatus is required, such as the apertometer devised by Abbe, or the simpler form suggested by Cheshire, as illustrated below, which can be used for dry objectives also. It gives a reading at sight without calculation, and consists of a circular disc of glass with a focussing mark on the upper surface and a series of concentric circles beneath, each circle corresponding to a variation of 0·1 N.A. The reading is taken by removing the eyepiece and counting the number of rings visible in the back lens of the objective. With a high-power objective, however, owing to the small size of the image, this is not an easy matter. To facilitate reading, therefore, a special eyepiece giving an enlarged image of the rings is supplied, which is inserted in place of the usual eyepiece.

FIG. 59.

Condensers.—The total numerical aperture can be measured on this apertometer, but the total aperture is not of so much consequence as the aplanatic aperture (see Appendix I., p. 78), which is best measured by illuminating with the condenser under trial a series of objectives of known aperture.

If the image of a lamp flame be focussed in the plane of the object by the condenser, the object, which should be mounted in balsam, being shifted slightly out of the optic axis, after focussing objective and condenser so that the light passes through the film of balsam only, it will be possible to find two objectives in the series, the back lens of one of which is completely filled with light, and the other only partially filled.

From which it is possible to say that the aplanatic aperture of the condenser is more than the numerical aperture of the one objective and less than that of the other ; the exact figure can only be arrived at by estimation. The more complete the series of objectives and the greater the skill and experience of the operator, the more accurate will be the determination.

Owing to the depth from back to front of the lamp flame, a

somewhat greater reading can sometimes be obtained by racking up the condenser slightly, without destroying the homogeneity of the illuminated area ; this should always be done, therefore, before making the final estimate.

Optical Performance of Objectives.

(a) **Spherical Aberration.**—Freedom from this defect must not be confounded with flatness of field ; the proof of good correction is to be found in the possibility of being able to focus every part of the field by use of the fine adjustment, not in an image which shall be equally sharp in every part of the field at one setting of the fine adjustment.

Flatness of field, however much it may be appreciated, and greatly as it is to be desired, is, unfortunately, impossible of association with objectives of fine quality. With low powers up to ½ inch it is generally obtainable for a considerable portion of the field, especially with objectives of small numerical aperture. The compromise which is to be made to secure it is not such in the low powers as to materially affect the general performance. It cannot, however, be given in objectives of medium and high power. A well-corrected objective inevitably has a curved field, and the more perfectly it is corrected, the more apparent does it become. This has become increasingly recognized, and it is now conceded that it is better to get the utmost perfection of definition in the central zone rather than that sharpness should be sacrificed to flatness of field. Flatness of field in any other than low-power objectives cannot, therefore, be expected except at the expense of inferior definition, and this to the critical worker would be intolerable.

(b) **Achromatism.**—Dr. Carpenter's old test for this correction —the examination of the cells in a thin section of deal—will give a very good idea of the colour corrections of objectives. For high powers, the markings on a frustule of the diatom *Pleurosigma formosum* are an excellent test. With the apochromatic objectives these come out quite black and white, while with those of the achromatic series any outstanding colour is at once revealed. Another method is the mercury test adopted by opticians. A small globule of mercury is placed on a slip of

ebonite, and a piece of whalebone or watch-spring is made to
snap on it, causing the globule to split up into numerous
particles of exceedingly minute size. These globules are then
examined with the objective, and can be illuminated by means
of a bare gas-jet, lamp, or daylight. Outstanding colour will be
revealed by the globules. A cover-glass of proper thickness must
be interposed when submitting lenses of considerable numerical
aperture to these tests.

A more permanent form of this test was embodied in the test
plate devised by Abbe, which originally consisted of six cover-
glasses of graded thickness, silvered on their under-surfaces, and
cemented to an ordinary object slip, across the silvered film of
each of which a number of coarse lines were ruled, the test
consisting in the observation of the image of the edge of the
silvered film.

This was later superseded by an improved form in which a
scaled wedge was substituted for the separate cover-glasses each
of a definite thickness, but such a test-plate requires considerable
experience to ensure reliable results, and must be considered
more an optician's tool than part of a microscopist's outfit.

Mechanical Movements.

The necessary tests for the mechanism of a microscope are
fairly obvious, more particularly if a high power be focussed.

All rack and pinion movements should move freely, but yet
not loosely, and should be free from back-lash ; that is to say,
that, having brought any such adjustment up to a certain point,
there should be no tendency for it to slip back on removing the
fingers, and there should be no slack to take up before a move-
ment commences.

There are, however, one or two points which are not so
obvious :

The fine adjustment mechanism cannot be tested until the
microscope is set up with the illuminant dead central ; for want
of this precaution, a misplaced mirror will often condemn an
instrument by showing an apparent lateral movement when using
the fine adjustment.

This adjustment, the most important and most delicate of
all the mechanical parts, should be tested, not only when the

instrument is vertical and inclined, but also in the horizontal position, more particularly if the microscope is likely to be used with a horizontal photomicrographic apparatus, as some types of fine adjustment are very sensitive to position.

The centring sub-stage should be tested, not only as to the possibility of centring the condenser to the optic axis of the instrument, but also to see that the excursion in various directions is approximately equal about that axis.

The draw tube should slide easily, and yet be held sufficiently tight by the collar into which it fits as not to slip under the weight of additional apparatus such as a drawing camera, or the inadvertent pressure of the cheek during observation.

All the above tests will serve to give one confidence in one's instrument rather than bring to light any serious discrepancy or falling off from standard if the microscope and accessories have been purchased from a reliable firm.

Tests of Adjustment.

We will now consider those tests which check the operator's use of the instrument; such tests are vastly more important to the beginner, as faulty manipulation is responsible for a thousand times more inefficient work than faulty instruments.

Ordinary, or Axial, Illumination.—Efficiency can be proved by removing the eyepiece and inspecting the back lens of the objective, the greater portion of which should be filled with a homogeneous or solid cone of light.

Very few objectives can be used advantageously with more than three-quarters of the area of the back lens thus illuminated, and even then only on selected objects. With most objectives and objects one must be satisfied with rather less than this, but the ¾-cone, nevertheless, remains the standard.

We need say nothing about position of mirror, centring of sub-stage, focussing of condenser, etc., because, if these are not correct, the even lighting of the back lens of the objective cannot be achieved; the one test, therefore, of efficient illumination is the observation of the back lens, and if this shows something wrong, one must go through the various steps detailed in Chapter IX. until the fault has been found and corrected.

Dark-ground illumination, as obtained with an ordinary condenser, can be tested in the same way.

After moving the object slightly to one side, so as to still allow the rays of light to pass through the medium in which the object is mounted or immersed, the back lens of the objective should appear practically dark, which proves that the stop is large enough.

By swinging out the dark-ground stop, closing the iris diaphragm of the condenser so that it is just visible on the edge of the back lens, substituting an objective of higher N.A., and swinging the dark-ground stop back into position, it can be seen, on again opening the iris diaphragm, whether the stop is central and whether its diameter is excessive, having regard to the correction of the condenser with which it is used.

Cover-Glass Correction.

The importance of making this correction has been referred to in both chapters dealing with ' Method of Use.'   We will now detail more fully how this correction can be applied in a systematic manner, either by rotating the collar sometimes fitted to the objective for the purpose, thus altering the relative position of the component lenses, or by alteration of tube-length.

One must bear in mind that the aim is to eliminate spherical aberration, which defect may be defined as a difference of focus between the central and marginal zones of an objective.   Hence the correct tube-length or the best position of the correction collar has been found when some strongly marked detail or outline of the object remains in exact focus under *any* change of illumination, say from a small to a large diaphragm opening beneath the condenser, or, better still, by changing the illumination from central to very oblique, these changes being made with great care, so as not to disturb the other adjustments.

The following process will be the safest and quickest: Start with the shortest tube-length, or, when there is a correction collar, with the position corresponding to the thickest cover-glass ; carefully focus some sharp outline with, say, a $\frac{1}{4}$ central cone, then change to a $\frac{3}{4}$-cone, or, better still, to very oblique light.   Unless the object—owing to an exceptionally thick cover-glass or a

very badly adjusted lens—is beyond the range of your adjustments, you will find evidence of under-correction—that is, the lens will have to be brought closer to the object with the wide cone, or oblique light, than with central light.

Gradually lengthen the tube, or turn the collar, repeating the above observation after each change, until all evidence of spherical aberration has disappeared; the instrument is then in correct adjustment within your own limits of vision.

It is advisable to start with the adjustment corresponding to the thickest cover, for the simple reason that this lessens the danger of running through the cover-glass and destroying the object, and possibly the front lens of the objective, when dealing with a lens of a short working distance.

The difference between an objective adapted to a 6-inch and that for a 10-inch tube is that in the latter case the back combinations of the objective are brought closer to the front lenses. This gives a slightly increased aperture. The majority of cover-glasses that are purchased and a large number of those used over commercial objects are more than 0·007 inch thick; 0·007 inch is a medium thickness of cover-glass, but the tendency is to use thicker ones. It will be found a great advantage to buy only such objectives as are corrected for a medium tube-length,* and having the rackwork before referred to fitted to the microscope tube, sufficient latitude would still be allowed if a thinner cover-glass were met with; but it would often be found necessary to close the draw tubes down to 6 or 7 inches, in order to get the best correction for the thick cover-glasses that are commonly used.

Test Objects.

It is very difficult to suggest a comprehensive set of test objects, although every microscopist who takes an interest in this side of the subject usually has some dozens. A mere list of names of objects is meaningless, and a description, more particularly of the detail to be seen, or which ought to be seen, under certain conditions, on the silicious skeletons of diatoms, would be equally so, condensed within the limits of the present

* Conrady has advocated that all objectives should be corrected for a tube-length of 8 inches, and with excellent reason, for such an arrangement would be a practical step in the solution of a difficult problem.

work. The following indications cannot but prove useful, however:

For correct setting up of the microscope:

The diatom *Pleurosigma angulatum* mounted dry on the cover-glass.

For experimental verification of the Abbe theory;

The diatom *Triceratium favus*. The primary structure of this triangular diatom is a coarse hexagonal mesh, giving, with a low power and the narrowest possible illuminating beam, a striking back lens picture of the spectra, which, with the central beam, build up the microscopical image.

Although the structure is coarse, these spectra are wide enough apart to be easily cut out singly by stops placed at the back of the objective, one of the most striking experiments thus rendered possible being the exclusion of all but two of the primary spectra, one on either side of the central beam; diaphragmed in this way, the structure is resolved into zebra-like stripes instead of hexagons, the rotation of the diaphragm making these stripes parallel first to one side and then another of the triangle forming the main outline of the diatom.

Owing to its close similarity to the much finer structure of *Pleurosigma angulatum*, it is of great assistance in understanding the possibilities of this useful test.

For colour correction:

Low power: Thin section of deal.

Medium power: The diatom *Navicula lyra*.

High power: The diatom *Pleurosigma formosum*.

For definition:

Low power: Proboscis of blowfly.

Medium power and high power: *Pleurosigma angulatum* mounted dry on the cover-glass.

For dark-ground illumination:

Low power: Strewn slide of Polycystina or Foraminifera.

High power: A minute scraping from the teeth in water showing the living bacteria always present in the mouth.

The exact determination of the character of the light employed as regards wave-length can best be determined with a direct vision spectroscope, but this is unnecessary except for advanced work.

APPENDIX I

GLOSSARY OF TECHNICAL TERMS

Aberration.—Any deviation of the rays of light when refracted by a lens which prevents the whole of the rays emanating from one point of the object being gathered together in a corresponding point in the image.

Achromatic.—Free from chromatic aberration.

Aplanatic.—Free from spherical aberration.

Apochromatic.—A term coined to designate a type of objective, the image given by which has a greater freedom from chromatic aberration than that given by the achromatic type (see p. 53).

Chromatic Aberration.—Want of sharpness in the image due to the unequal refraction of the various wave-lengths—that is, colours—of which the light by which the object is seen is made up.

Chromatic Over-Correction.—A term applied to a lens when rays towards the red end of the spectrum are best corrected.

Chromatic Under-Correction.—A term applied to a lens when rays towards the blue end of the spectrum are best corrected.

Compound Microscope.—An instrument in which the image given by one lens or series of lenses is picked up and still further magnified by another lens or series of lenses.

Cover-Glass.—The thin glass (average thickness, 0·008 inch) with which the object is covered.

Dark-Ground.—A method of illumination by which the object appears self-luminous on a field which receives no direct light, and is therefore dark or black by contrast.

Diaphragm.—This is understood in optical instruments to be a circular opening in a plate, the unpierced portion of which serves to cut off the marginal rays of a beam of light. The adjustable pattern fitted to condensers called an 'iris' diaphragm is now so universally used that the word 'iris' is often used by itself as equivalent to diaphragm, although a microscopist still speaks of stopping down a condenser—a survival from the time when separate plates or stops, each with a different sized aperture, were used for this purpose—the word 'stop' being now reserved for the discs used for cutting out the central beam and thus obtaining dark-ground illumination.

Diffraction.—See p. 4.

Draw Tube.—The tube, adjustable in length, which carries the eye-piece.

Eye-Lens.—The upper lens of an eyepiece.

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Eyepiece.—The lens, or combination of lenses, nearest the eye in a compound microscope ; sometimes referred to as an ' ocular.'

Field.—A contracted form of ' field of view '—that is, the disc of light visible when looking into the eyepiece, within the bounds of which the object is seen.

Field Lens.—The lower lens of an eyepiece.

Mechanical Stage.—A stage carrying the object which has movements in two directions at right angles to one another—*i.e.*, lateral and back to front—built into the instrument (see Fig. 41, p. 36) ; or an attachment with similar movements which clips the object-slip and moves it over the surface of the fixed stage (see Fig. 40, p. 35).

N.A. = Numerical Aperture.—A measure of the resolving power of a lens (see p. 5).

Nosepiece.—The end of the tube into which the objective is screwed.

O.I. = Optical Index.—See p. 5.

Objective.—The lens, or combination of lenses, nearest the object in a compound microscope.

R.I. = Refractive Index.—See p. 2.

Ramsden Disc.—The plane at which the rays cross on emergence from the eyepiece. The distance of this plane from the eye lens varies with the power of the eyepiece.

Resolution.—The revelation of the ultimate structure of an object.

Revolving Nosepiece.—An adapter carrying two or three objectives, any one of which can be rotated into position as required. See Fig. 39, p. 34, showing one in position on the microscope.

Secondary Spectrum.—In an achromatic lens the chromatic aberration is corrected for the brightest (yellow or green) rays of the spectrum, and the pronounced colour shown by uncorrected lenses is in consequence removed. A stricter examination, however, shows that rays of a different colour are not brought to the same focus, for owing to the fact that flint-glass, as compared with crown-glass, disperses the more refrangible rays relatively too much, and the least refrangible relatively too little, a peculiar *secondary* spectrum results from the achromatic combination, the rays corresponding to the brightest apple-green part of the ordinary spectrum being very closely united and focussed nearest the combination, whilst the other colours focus at increasing distances *in pairs*, yellow being united with dark green, orange with blue, red with indigo. The composite effect of these colours is best seen with oblique light, causing dark objects to have apple-green borders on one side and purple ones on the other.

Semi-Apochromatic.—In achromatic microscope objectives of the older type, chromatic defects that are worse than the secondary spectrum

are caused by spherical aberration of the coloured rays, the spherical aberration being corrected for the brightest part of the spectrum only. Objectives made entirely of glass, and therefore showing the secondary spectrum, are called semi-apochromatic when the spherical aberration is corrected practically for all colours.

Slip.—Practically all objects are mounted, temporarily or permanently, on slips of glass 3 inches long by 1 inch wide, such a mount being usually referred to as 'the slip.'

Spectrum.—The band of colours produced by splitting up white light by means of a prism (Fig. 2, p. 2) or a finely ruled grating (see under Diffraction, p. 4).

Spherical Aberration.—Rays of light passing through the marginal portion of a lens come to a focus nearer to the lens itself than those rays which pass through the centre of the lens, and the interval between the focal points of rays which pass through the marginal and the central parts of that lens is the spherical aberration. In compound lenses this spherical aberration can be corrected for one or more special rays, and a lens so corrected is called aplanatic. It is only truly aplanatic for the particular rays for which it has been accurately corrected.

Spherical Over-Correction.—A condition in which the lens unites the marginal rays at a greater distance than the central rays.

Spherical Under-Correction.—The reverse of above. For illustration, see Fig. 28, p. 23.

Spherical Zones.—In objectives of considerable aperture the intermediate rays may show decided spherical aberration, although the central and marginal rays are united. This defect is meant when spherical zones are spoken of. The degree to which spherical zones are corrected determines chiefly how large a cone of illumination and how deep an eyepiece an objective will bear before 'breaking down.' A high degree of correction for spherical aberration and spherical zones must accompany the reduction of chromatic defects before terms such as 'semi-apochromatic,' and especially 'apochromatic,' can be applied to a lens.

Stage.—The table or platform on which the object to be examined is placed.

Stop.—A diaphragm with an opaque centre (see Diaphragm).

Sub-Stage.—The mechanism fitted beneath the stage to carry the illuminating apparatus.

Zone.—A narrow annulus between centre and periphery at any distance from the centre.

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APPENDIX II

OFFICIAL GAUGES AND SPECIFICATIONS

The Royal Microscopical Society Standard Gauges.

EYEPIECES.*

    Internal diameter of draw tube :
      Small size ...   .   0·917 in. = 23·3 mm.
      Large size ...   .   1·27   in. = 32·25 ,,

SUB-STAGE.

    Internal diameter of fitting   ...   1·527 in. = 38·786 mm.

OBJECTIVE SCREW-THREAD.

      Diameter   ...   .   0·8000 in.
      Core diameter   ...   ...   0·7644 ,,
      Effective diameter   ...   ...   0·7822 ,,
      Pitch   ...   .   0·0277 ,,
      Number of threads per inch   ...   36.
      Form Whitworth Screw.

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   * The standards for eyepieces adopted by the R.M.S. in 1899 were four in number :
                 No. 1.—0·9175 in. = 23·300 mm.
                 No. 2.—1·04   ,, = 26·416 ,,
                 No. 3.—1·27   ,, = 32·258 ,,
                 No. 4.—1·41   ,, = 35·814 ,,

    At the present time practically only two eyepiece standards are in general use, viz., the Nos. 1 and 3, and the Council recommends that these should be known respectively as the small and large size R.M.S. Standard Eyepieces.

The British Science Guild Specifications.

TYPE I.—A cheap instrument for the use of students.

1. *Stand.*—The modified Continental type, with jug handle; with spiral rack and pinion coarse adjustment.

2. *Tube.*—Short, with graduated draw tube, allowing for length of nosepiece; the available tube-length should be from 140 mm. to 180 mm.

3. *Fine Adjustment.*—Lever type; lateral milled heads.

4. *Stage.*—Large, square, fair-sized opening, provided with clips of simplest type, having points of contact level with equator of stage.

5. *Mechanical Stage.*—Not required, but provision made for its later addition.

6. *Sub-Stage Condenser.*—Abbe type, accurately centred and fitted with iris diaphragm.

7. *Nosepiece.*—Dust-proof. Position of objectives marked on nose-piece.

8. *Objectives.*—Two, of focal length 16 mm. and 4 mm. (or their equivalents), the latter with good working distance, both engraved with focal length, N.A., tube-length, and the magnification at the distance for which the objective is corrected expressed by X . . .

9. *Oculars.*—Two, of focal length 40 mm. and 25 mm. Each ocular should be engraved with the initial magnification, the focal length in mm., and with a magnification number arrived at by dividing 250 by that focal length and expressed by X . . .

The microscope should be capable of carrying the additions required for more advanced work.

TYPE II.—A good instrument for advanced pathological work.

1. *Stand.*—Modified Continental jug-handle type, with spiral rack and pinion coarse adjustment.

2. *Tube.*—Short, with graduated draw tube; the scale to allow for length added to the tube by the changing device (viz., nosepiece). The available tube-length should be from 140 mm. to 180 mm.

3. *Fine Adjustment.*—Lever type. Lateral milled heads of small diameter, but long milled surface.

4. *Stage.*—Large. Square, with sufficient room to accommodate a large Petri dish. Clips of simple description, having points of contact level with equator of stage.

5. *Mechanical Stage.*—(a) Detachable type: rack and pinion covered through entire length and actuated by milled heads of small diameter, but with long milled surface. Must work smoothly, and permit of examination of at least 2 inches of a 3-inch slide. (b) Built-in type.

6. *Sub-Stage Condenser.*—Abbe type. Rack and pinion, centring, iris diaphragm.

7. *Triple Nosepiece.*—Dust-proof; position of the three objectives to be marked on nosepiece.

8. *Objectives.*—Three, of 16 mm., 4 mm., and 3 or 2 mm. oil immersion. Colour of mount of immersion lens to differ from others, and preferably to be black. Each objective should be engraved with the tube-length, the N.A., and the magnification at the distance for which the objective is corrected expressed by X . . .

9. *Oculars.*—Two, of focal length of 40 mm. and 25 mm. Each ocular should be engraved with the initial magnification, the focal length in mm., and with a magnification number arrived at by dividing 250 by that focal length and expressed by X . . .

TYPE III.—An instrument designed especially for research work, the character of this to depend on the individual requirements of the different workers.

1. *Stand.*—With spiral rack and pinion coarse adjustment, and to be equally stable in vertical or horizontal positions. The centre of mass should be kept as low as possible consistent with freedom of access to the sub-stage.

2. *Tube.*—Minimum diameter of body tube to be 50 mm. internally, with double draw tube, so that either long or short tube objectives can be used. Second draw tube to be 100 mm. long, and both draw tubes to be graduated in mm.

3. *Fine Adjustment.*—Lever type. Lateral milled heads on both sides of stand, of small diameter, but with long milled surface. Fine adjustment must be graduated.

4. *Stage.*—Circular rotating, with centring screws and provided with a clamp. The mechanical part should be graduated with verniers and be detachable, and so fitted that when removed a plane surface is left. As an alternative, it should be built in with separate and independently operating milled heads.

5. *Objective Changing.*—Should have sliding objective changers, which should be provided with centring screws.

6. *Sub-Stage.*—Should have centring screws. Rack and pinion to be exceedingly well fitted and made. Sub-stage iris diaphragm should be rotatable, and should have rack-work lateral motion.

7. *Condenser.*—An achromatic immersion condenser which can be used dry.

8. *Dark-Ground Illuminator.*—Type in which there are spherical reflecting surfaces. Some device in upper surface which, without interfering with the satisfactory working of the accessory, will indicate the axial centre.

9. *Objectives.*—The following battery of lenses should form the foundation, which would, of course, need addition for the particular research work engaged upon : 16 mm., 8 mm., 3 mm., or 2 mm. focal length. Each objective should be engraved with the tube-length, the N.A., and the magnification at the distance for which the objective is corrected, expressed by X . . . Mounting of immersion lenses to be distinctive, preferably black.

10. *Oculars.*—Two to form the basis of equipment, of 40 mm. and 25 mm. focal length. Each ocular should be engraved with the initial magnification, the focal length in mm., and with a magnification number arrived at by dividing 250 by that focal length and expressed by X . . .

APPENDIX III

MICROSCOPICAL SOCIETIES AND CLUBS

The Royal Microscopical Society.

(*Established* 1839. *Incorporated by Royal Charter*, 1866.)

20, HANOVER SQUARE, LONDON, W. 1.

President: PROFESSOR FREDERIC J. CHESHIRE, C.B.E., F.INST.P.

Hon. Secretaries { JOSEPH E. BARNARD, F.INST.P.
{ JAMES A. MURRAY, M.D.

THE Society was established in 1839 for the promotion of micro-scopical and biological science by the communication, discussion, and publication of observations and discoveries relating to (1) improve-ments in the construction and mode of application of the microscope, and (2) biological or other subjects of microscopical research.

It consists of ordinary, honorary, and ex-officio Fellows of either sex.

Ordinary Fellows are elected on a certificate of recommendation signed by three ordinary Fellows,* setting forth the names, residence, and qualifications of the Candidate, of whom the first proposer should have personal knowledge. The certificate is read at two general meet-ings, the candidate being balloted for at the second meeting.

The admission fee is £2 2s., payable at the time of election; and the annual subscription is £2 2s., payable on election and subsequently in advance on January 1 in each year, but the annual subscriptions may be compounded for at any time for £31 10s. Fellows elected at a meeting subsequent to that in February are only called upon for a pro-portionate part of the first year's subscription. The annual subscrip-tion of Fellows permanently residing abroad is £1 11s. 6d., or a reduction of one-fourth.

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* Forms of proposal for Fellowship, and further information, may be obtained on application to the Secretary, 20, Hanover Square, London, W. 1.

Honorary Fellows (limited to 50), consisting of Fellows eminent in microscopical or biological science, are elected on the recommendation of five ordinary Fellows and the approval of the Council.

Ex-officio Fellows (limited to 100), consisting of the Presidents for the time being of any societies having objects in whole or in part similar to those of the Society, are elected on the recommendation of ten ordinary Fellows and the approval of the Council.

The Council, in whom the management of the property and affairs of the Society is vested, is elected annually, and is composed of the President, four Vice-Presidents, Treasurer, two Secretaries, and twelve other ordinary Fellows.

The meetings are held on the third Wednesday in each month from October to June, in the Lecture Hall, at 20, Hanover Square, W. (at 7.30 for 8 p.m.). Visitors are admitted on the introduction of Fellows. The business of the meetings includes the reading of papers, the exhibition of microscopical objects and apparatus (usually on view by 7.30 p.m.), lantern demonstrations, and discussions.

SECTIONAL MEETINGS.

During the session additional and less formal meetings are held in the Society's library, and are devoted to exhibits, communications, and discussions.

The Biological section meets in the Library on the first Wednesday in each month at 7 for 7.30 p.m. Hon. Secretary : Mr. J. Wilson, ·3, West Park Road, Kew Gardens.

The Leather Industries section meets occasionally at 7 for 7.30 p.m. Hon. Secretary : Dr. S. H. Browning, 22, Harley Street, W. 1.

The Metallurgical section meets occasionally. Hon. Secretary : Mr. F. Ian G. Rawlins, White Waltham Grove, near Maidenhead, Berks.

A Paper Industries section is in course of formation, and will deal with researches relating to timber, wood pulp, paper, etc. Those who are interested in the subject and willing to assist are asked to communicate with Mr. James Strachan, F.Inst.P., 74, Blenheim Place, Queen's Cross, Aberdeen.

THE LIBRARY.

The Society's rooms, in which are housed the reference and lending library, the extensive and illustrative collection of instruments and apparatus, and also the unique collection of type slides, etc., are open for the use of Fellows on all week-days (except Saturdays and public holidays), from 10 a.m. to 5 p.m., with the exception of four weeks during August and September. The library and rooms of the Society are also open for the use of Fellows on Wednesday evenings, other

than meeting evenings, from 6 to 9 o'clock, except during the vacations. Situated in the centre of the West End, they are easily reached from Oxford Circus, Bond Street, Piccadilly Circus, or Dover Street Tube Stations.

The Fellows residing outside the London area, but within the British Isles, are enabled to obtain by post any of the volumes in the Society's Library (of which there is a printed catalogue) with the exception of a few of the rarest works, and as the Society is also a subscriber to Lewis's Medical and Scientific Library, practically any recent scientific work can be obtained for a Fellow requiring it.

THE JOURNAL.

The Society's Journal is edited by Professor John Eyre, M.D., M.S., F.R.S.E., and Charles Singer, M.A., M.D., F.R.C.P., with the assistance of the Publication Committee, and J. Arthur Thomson, M.A., LL.D., F.R.S.E., Regius Professor of Natural History in the University of Aberdeen; J. E. Barnard, F.Inst.P., A. N. Disney, M.A., B.Sc., J. Bronté Gatenby, B.A., B.Sc., D.Phil., F. Ian G. Rawlins (Fellows of the Society); and A. B. Rendle, M.A., D.Sc., F.R.S., Keeper, Department of Botany, British Museum. It is published quarterly. All Fellows are entitled to a copy, and it is also sold to non-members, at an annual subscription of 42s. post free. It contains :

1. The transactions and proceedings of the Society.

2. A summary of current researches relating to zoology and botany (principally invertebrata and cryptogamia) and microscopy contained in the leading scientific journals of the world.

The Quekett Microscopical Club.
(*Founded* A.D. 1865.)

President: D. J. SCOURFIELD, F.R.M.S.

Hon. Secretary: S. R. WYCHERLEY, F.R.M.S.

The Quekett Microscopical Club, founded principally by the late Dr. M. C. Cooke, who presided at its inaugural meeting on July 7, 1865, has, according to the prospectus then issued, 'been established for the purpose of affording to microscopists in and around the Metropolis opportunities for meeting and exchanging ideas without that diffidence and constraint which an amateur naturally feels when discussing scientific subjects in the presence of professional men.'

The ordinary meetings of the Club are held on the second Tuesday in each month from October to June inclusive, when communications relating to microscopic subjects are read and discussed, new instruments and apparatus exhibited and described, and interesting objects displayed. Meetings for 'conversations' and exhibition of objects are held on the fourth Tuesday of each month during the year, and on the second Tuesday of the months of July, August, and September.

During the summer months excursions are held on Saturday afternoons to various well-known collecting grounds around London, to afford members valuable facilities for becoming acquainted with the haunts and habits of living organisms under experienced collectors and guides.

The Club possesses an extensive library of scientific works and a large collection of mounted microscopic objects for lending out to members.

The annual subscription is 10s., no entrance fee, and members receive a copy of the Club's *Journal of Proceedings* as published either once or twice in the year. The Club has over 500 ordinary members and a few honorary members, distinguished for their work in the scientific world.

The meetings are held at the Rooms of the Medical Society of London, 11, Chandos Street, Cavendish Square, W. 1, to which all correspondence should be forwarded, addressed 'The Secretary.'

The Photomicrographic Society.

President: JOSEPH E. BARNARD, F.R.M.S., F.INST.P.
Secretary: J. G. BRADBURY, F.R.M.S., 1, Hogarth Hill, Finchley Road, Hendon, N.W. 11.

The Photomicrographic Society was founded in 1911 by a small group of microscopists and photographers, the majority of whom were already Fellows or members of the Royal Microscopical Society and the Royal Photographic Society, with the object of banding together those workers interested in the recording of microscopical observations by photography. Their opinion at the time, amply confirmed in later years, was that the use of photomicrography in those branches of research usually connected with the microscope would be followed by wide extension to commercial and other branches, and at the present day the Society has on its roll of members many who are connected with medical and biological research, engineering, public health, and those trades and professions which use the microscope and have to record the result of their observations.

To the efforts of its first President, the Rev. F. C. Lambert, F.R.P.S., the foundation of the Society was mainly due, and others who followed him in this office were the late T. E. Freshwater, F.R.M.S., F.R.P.S., G. Ardaseer, F.R.P.S., J. E. Barnard, F.Inst.P., F.R.M.S. (past President of the Royal Microscopical Society), Dr. G. H. Rodman (now President of the Royal Photographic Society), F. Martin Duncan, F.R.M.S., F.R.P.S., F.Z.S., Commander M. A. Ainslie, F.R.A.S., F.R.M.S., and Dr. Duncan J. Reid, F.R.M.S. Mr. Barnard is now President for the second time, and to his help, especially in its early days, the Society owes a large measure of its success.

It is interesting to note that the first member to be actually elected was the late Dr. E. J. Spitta, the well-known author of what may be regarded as the first standard book on photomicrography.

From the first the Society was warmly welcomed, and has increased in numbers year by year. It has always recognized that the first essential to success in photomicrography is a thorough understanding of microscopic technique, especially as regards correct methods of illumination. Following upon this, its objects have been the study of methods of mounting and staining microscopic objects, the use of light filters, etc., and the improvement of microscopic and photographic apparatus to the same end. A marked feature has been the attention paid by individual members to the designing of new and improved apparatus and improvements to illuminants.

The Society's meetings are held at King's College, Strand, at 7 p.m., on the second and fourth Wednesdays in the month from October

to May inclusive, the first meeting in each month being a lecture or demonstration; the second meeting, of an informal character, being devoted to discussion, demonstration, and exhibition of members' photomicrographic work. Visitors are heartily welcomed on the lecture evenings.

Examples of the Work of Members.

For examples of the work of the members, see the frontispiece of this volume, the photomicrographs of which were contributed by the following :

(A). Diatom, *Asteromphalus arachne*, × 840. E. A. Pinchin, F.R.M.S.

(B). Blood parasites, *Trypanosome brucei*, × 1,040. Dr. Duncan J. Reid, F.R.M.S.

(C). Section of Nummulite in limestone, × 9. C. H. Caffyn, F.R.M.S.

(D). Surface of plain Portland cement showing blowholes compared with Portland cement mixed with a commercial preparation to render it damp-resisting and homogeneous, × 20. J. G. Bradbury, F.R.M.S.

(E). Structure of an electric weld, of the ' V butt ' type, between two $\frac{5}{4}$-inch mild steel plates, of the quality used in shipbuilding. Molten steel, in three layers, has been filled into the V-shaped space between the ends of the two plates. The view shows the differences between the characters of the several layers of metal, and also the effect on the plate of the intense heat of the electric arc. It will be seen that the upper part of the first layer and the whole of the second layer have been ' refined ' by the heat, while the bottom of the first layer and the whole of the third layer retain their original course structure. Such low-power views—in this instance only two magnifications—are particularly serviceable for studying welds of this type. Under intermediate powers, 100 to 200 magnifications, many of the characteristic structural features are plainly revealed ; but to show those characteristics on which depend the brittleness or toughness of the weld, higher magnifications of 500 to 1,000 diameters are desirable. Professor B. P. Haigh, D.Sc., M.B.E.

(F). Eggs of spider, × 10. W. H. Baddeley, F.R.M.S.

The Manchester Microscopical Society.

(Affiliated to the Royal Microscopical Society.)

President : PROFESSOR F. E. WEISS, D.Sc., F.R.S., F.L.S.

Hon. Secretary : WILLIAM DIXON, Broadwater, 48, Pine Road, Didsbury, Manchester.

This Society was founded in 1880 to associate residents in the city and its suburbs interested in microscopy and natural history.

Its members number over two hundred, including honorary, corresponding, and ordinary members. Ladies are eligible for election.

Subscription.—Entrance fee, 5s.; annual subscription, 8s. 6d.

The meetings are held in the rooms of the Literary and Philosophical Society, 36, George Street, on the first Thursday of the month, except in July and August, when there are no meetings, and September, when the meeting is held on the third Thursday.

All meetings commence at 7.0 p.m.

A Mounting Section was formed in 1882, with the object of holding demonstrations in the most approved methods of preparing and mounting microscopical objects, and in practical biology and microscopy. Membership is confined to members of the Society. Subscription, 5s. per annum. The meetings are held on the third Thursday in the month, except during July, August, and September.

An Extension Section was founded in 1898, its object being to bring scientific knowledge, in a popular form, before other societies who are unable to pay large fees to professional lecturers. The work is entirely voluntary and gratuitous on the part of the members.

The Society possesses an extensive library, a large collection of microscopical slides, and several microscopes, with the necessary accessories, all of which are accessible to members.

Rambles are arranged for most Saturdays in the summer, for the collection of various microscopical objects, 'pond life' being the most popular.

The Society issues a monthly circular to members, and publishes its Annual Report and Transactions in September. The latter is obtainable from the Secretary at the address given above, the charge to non-members being 1s. 6d. (by post 1s. 7½d.).

NOTE.—A number of provincial Natural History Societies have a microscopical section, particulars as to any one of which can be obtained by communicating in the first instance with the Secretary of the Royal Microscopical Society, 20, Hanover Square, London, W. 1.

PART II

THE MICROSCOPE AND THE SCIENTIST

CHAPTER XII

THE MICROSCOPE IN MEDICINE—INTRODUCTION

By JOSEPH E. BARNARD, F.R.M.S., F.Inst.P.
Past President of the Royal Microscopical Society ; President of the Photomicrographic Society.

In no branch of science is the use of the microscope so widespread as in that department known as medicine, using the term in its widest sense to cover all its allied branches; no apology, therefore, is needed for the recapitulation of various details already dealt with in the preceding pages, changing our point of view from the general to the particular.

In medicine the instrument is used for educational, diagnostic, and research purposes, the two former being but the offspring of the last, for the research of to-day, the pushing on into the unknown, becomes the ascertained fact of to-morrow, to be utilized educationally to teach a truer conception of the functions of the various organs of the human body and of other organisms, whether parasitic or otherwise, affecting its health, or clinically to aid in the diagnosis of disease.

If, therefore, one would understand what is meant by the comprehensive term 'medical research,' as pursued with the aid of the microscope, one cannot do better than peruse the two following chapters, every fact stated therein having once formed the subject of long and patient enquiry, the elucidation of which has opened up still further avenues for attack on problems as yet unsolved, some of which are enumerated at the end of Chapter XIII.

It is the province of research not only to discover facts by means of every appliance and refinement of technique possible, but, when found, to suggest the means by which details, so

PLATE II.

FIG. 60.—SAC. PASTORIANUS.
STAINED. × 675.

FIG. 61.—SAC. PASTORIANUS.
DARK GROUND. × 900.

FIG. 62.—SAC. PASTORIANUS.
ULTRA VIOLET LIGHT. × 1125.

FIG. 63.—B. ANTHRACIS.
STAINED. × 1125.

FIG. 64. — B. ANTHRACIS.
DARK GROUND. × 1125.

FIG. 65.—B. ANTHRACIS.
ULTRA VIOLET LIGHT. × 1350.

elusive that they have been previously wrongly interpreted, or even passed over altogether, can be brought within the range of the ordinary microscope and the routine microscopist. It may be by devising some new stain or reagent, which will differentiate structures apparently homogeneous when treated by older methods, or some new appliance, such as the high-power dark-ground illuminator, by means of which living bacteria can now not only be demonstrated, but specific differences recognized by anyone after a few lessons, the ability to do so previous to its introduction having rested with a few microscopists possessing special knowledge and an unusually complete optical equipment.

We will now proceed to specify the most suitable equipment for these three classes of work.

Educational Equipment.

For educational purposes, such as the demonstration to the student of the histological detail dealt with in Chapter XV., a simple type of instrument is to be preferred. All makers now produce a students' microscope which is simple in design and has none of the extra equipment that is necessary for diagnostic or research work. Further, the instrument must be inexpensive and yet must have sufficient optical equipment for the work in view.

The base is usually of the horse-shoe pattern, the stage is plain and simply fitted with clips to hold the object, no mechanical stage being provided, and unless bacteriological work is contemplated, the objectives and eyepieces are confined to a low and medium power. A good coarse adjustment is essential, together with a fine adjustment which is not too slow in action. Most students wish for a mechanical stage, so long as it does not unduly increase the cost of the instrument, but, as a matter of fact, it is doubtful if it is advisable for them to have it in any case. There is no better means of acquiring the delicacy of touch requisite for efficient manipulation than to move a specimen on the stage of a microscope without mechanical aid. Further, a student should learn to completely search a specimen without missing any part of it before he has recourse to a mechanical stage. A sub-stage with an Abbe illuminator should be provided,

and it is recommended that this should have some arrangement for altering its position in relation to the object. A plane mirror only is necessary, as the student should realize that the mirror is really only an arrangement for altering the direction of the incidence of the light in relation to the optical axis of the microscope. The illuminant provided is usually an ordinary electric lamp, but if this is so, it should be contained in a fine ground glass bulb. In front of the bulb, and nearly in contact with it, should be placed a piece of black metal or cardboard with a circular hole in it, about ¾ inch in diameter. This circular hole, then, in effect, becomes the light source, and the image of it should be projected centrally up the microscope body tube.

Even the simplest microscopes are provided with draw tubes so that the body tube of the instrument may be lengthened or shortened, within considerable limits, at will. The student should realize that this is not an arrangement for altering magnification, but is provided for the purpose of correcting the errors that arise from the use of different thicknesses of cover-glass, this factor influencing the image when medium-power objectives are used. This is a point to which, under ordinary laboratory conditions, attention is rarely directed; but it is in reality about the most important adjustment that a student is called upon to make while using a microscope after the obvious one of focussing. It is often necessary to point out that under no condition can a good image be obtained when an object is simply mounted on a slide without a cover-glass and is being examined with, say, a ⅙ inch objective. The examination of preparations without a cover-glass is only too common in laboratories, and is the main cause of so many objectives of medium power being described as 'indifferent.' The coarse adjustment, even on a student's stand, should be sufficiently well made to bring any object into view, whether he is observing it with a high or low power. That is to say, that even with an oil immersion objective he should find it possible to get his object into the field of view, not in accurate focus, but distinctly in view. Then recourse should be had to the fine adjustment to finally focus the image, and it is only for this purpose that the fine adjustment should be used.

A student's microscope should be of such construction that additional appliances, which may be necessary at later and more advanced stages of his work, can be added without difficulty. Should he, therefore, require to do diagnostic work, the only addition to the stand that is really necessary is a mechanical stage. If the microscope has a square stage of fairly large size, then a mechanical stage of the detachable variety is to be preferred. In most cases these are so constructed that a specimen on an ordinary slide may be examined over the whole of its surface, many of them permitting an area 3 inches by $1\frac{1}{2}$ inches to be completely searched. The main point is that the object should be moved over the stage in such a way that it remains in contact with it the whole time, and the motions imparted to it should be at right angles to one another. The student should be instructed in the use of low and medium power objectives before he is allowed to do any observation with an oil immersion —a provision that is rarely borne in mind. It cannot be too early recognized that while it is not at all difficult to see an image in the microscope, it is much more difficult for the observer to so adjust the instrument as to get the best possible image and to interpret accurately what he sees.

Two oculars are sufficient for either students' purposes or for diagnostic work. One of these should be of 42 mm. focal length, with an initial magnifying power of about six times, and another of 25 mm. focal length, with an initial magnifying power of ten.

Diagnostic Equipment.

The essential addition to a microscope of the students' type, if it is to be used for diagnostic work, is the provision of a $\frac{1}{12}$ inch oil immersion objective.

It is to be regretted that this particular focal length is still almost exclusively adhered to in laboratories, but it is probable that at no distant date it will be realized that an objective of longer focus — for instance, a $\frac{1}{7}$ or $\frac{1}{8}$ inch oil immersion (see Optical Index, p. 5)—has great advantages for diagnostic work. Except for the fact that an oil immersion objective has a shorter working distance than a medium-power dry lens, it is really an easier objective to use than some dry objectives of considerably

greater focal length. This must not be taken to mean that the interpretation of the image formed by an oil immersion objective is easier than with a dry objective.

Diagnostic work is almost entirely confined to the branches of bacteriology and pathology, and diagnosis is usually dependent on the differentiation of structure which results from staining reactions. It follows, therefore, that the microscope must be so used as to most easily show these differences in colour, more especially in cases where the variation of structure is only indicated by a delicate gradation of tint. To diagnose pathological changes which result in colour *differences* is usually not difficult; in fact, in most staining reactions of this description the colours are well marked. For example, in the identification of the tubercle bacillus in sputum, the bacillus appears a bright red, while all other organisms are stained a deep blue. In some cases, however, differentiation is more subtle, and it is under those circumstances that it is important that the source of light which is used should approximate as nearly as possible to daylight. Daylight itself is, under certain circumstances, quite satisfactory, but owing to its variability it can hardly be relied on, at least in this country. If an electric lamp is used it should be one which gives as white a light as possible, so that the more modern type of lamp with metal filament, in which the globe is gas filled, is preferable.

Practically all stained preparations can be shown best with a wide cone of light. It is advisable, therefore, that the cone of illumination shall be as large as possible, this, of course, being largely dependent on the character of the sub-stage condenser. For this reason, when setting up the microscope for the examination of stained preparations, the eyepiece should always be removed to ascertain whether the greater part of the back lens of the objective is actually filled with light or not. With eyepiece removed one can see at a glance whether the full effective objective aperture (see p. 54) is being used; usually with the higher powers there is a central brightly illuminated portion surrounded by a more or less dark area, sometimes irregular in form. The sub-stage condenser should be moved up and down until the bright central portion is as large and evenly illuminated as possible.

It is unfortunately true that microscopic work for purposes of diagnosis is not now carried out with any considerable care, a condition of things that did not greatly matter so long as the staining reactions that were relied on were comparatively gross in character; but the introduction of more delicate staining processes in recent times renders it imperative that not only more care, but more understanding of the possibilities of the instrument, should be brought to bear on the decision. It is in many cases now realized that the staining of an organism deeply, so that it is evident simply as a silhouette, is not of necessity the best method. To observe delicately stained organisms satisfactorily involves much more care in the use of the microscope, more accurate setting up of the illuminating system, and the provision of an arrangement by which the actual intensity of the light can be varied at will. The latter point is of considerable importance, and it is probable that the most satisfactory light modifier is that which can be made by half-silvering or semi-platinizing a surface of glass. Such light modifiers can now be obtained commercially, and will no doubt become much more general in use when their value is fully realized.

Research Equipment.

The type of outfit required for research work is dependent on the purpose in view. A considerable amount of research work can be carried out with any good microscope that has been found satisfactory for purposes of diagnosis. At the same time, for work of the highest class it is necessary to have a stand that is thoroughly well made in every respect—in fact, the best that can be bought—with a fine adjustment that is delicate and certain in action, and with sub-stage illuminating apparatus of a considerably higher class than for any other purpose.

There are two electrical illuminants at present available which are suitable for research work of the most exacting character. These are the Pointolite lamp made by the Edison Swan Electrical Company, and a mercury vapour lamp such as is now provided by the Silica Syndicate. The Pointolite lamp provides a small circular illuminant of uniform intensity. It is advisable to have in front of the illuminant an aplanatic condensing

7

system of 3 to 4 inches focal length, which will give a parallel beam sufficient in size to all but cover the surface of the mirror of the microscope (Fig. 51, p. 44). A sub-stage is desirable which is provided with screws, so that it can be centred accurately to the optic axis of the microscope, fitted with an aplanatic condenser, which can be used, if required, as an immersion one, so that when using apochromatic objectives of highest N.A. the full aperture of the objective may be brought into use. The purpose of such a condenser is to evenly and completely fill the objective with light. If the subsidiary condensing lens which is in place near the illuminant is provided with an iris diaphragm, it forms a very convenient method of altering the size and the intensity of the illumination without interfering with the conditions which should be established.

The mercury vapour lamp is essentially different in principle from a Pointolite, as the luminous source is, in this case, mercury vapour, which fills a tube, preferably of quartz. In this case the light source is larger and is not perfectly regular in its intensity over the whole area. It is therefore advisable to have in front of the lamp a screen in which a hole is made, exposing a circle of about $\frac{1}{2}$ inch in diameter of the luminous portion of the tube. A condensing system may then be put in front of this aperture, throwing a parallel beam on to the mirror of the microscope, or the luminous tube can, if preferred, be used directly as a source of light.

The advantage of the mercury vapour lamp is that it emits hardly any—in fact, for all practical purposes, no red radiations at all. The result is that its mean wave-length is less than that of any other illuminant; and as it is well known that one of the factors determining resolution in the microscope is the wave-length of the light that is used, it follows that resolution is, other things being equal, higher with a mercury vapour lamp than with any other light source. It has the further advantage that it consists of yellow, green, and blue radiations chiefly, and that these radiations are emitted from three bright lines in the spectrum. It is only necessary, therefore, to screen off the radiations it is desired to eliminate, and that transmitted consists of truly monochromatic light of one particular wave-length.

It is probable that this source of light will become a very general one for research work of the highest class, as it has characteristics which put it above all others.   At the National Institute for Medical Research it is now regarded as the final court of appeal when any delicate structure has to be elucidated.

High-Power Dark-Ground Illumination.

The equipments described above are intended for the examination of stained preparations, or, at least, those preparations in which axial light is used.   It is, however, self-evident that in medical work at the present time the method known as dark-ground illumination is becoming ever more important.   In the identification of such organisms as *Spirochæta pallidum* the use of a method in which the organisms are rendered self-luminous · is exceedingly important.   For this work any good microscope which would be suitable for research or diagnostic purposes may be employed.   The essential differences of the equipment are that a light source of suitable intensity must be used, and that a condenser, which is usually known as a dark-ground illuminator, is substituted for the ordinary sub-stage condenser.

The most satisfactory light source for dark-ground work is undoubtedly the Pointolite lamp already referred to.   It has been customary to recommend the use of small arc lamps for this purpose, but it cannot be too strongly insisted on that such unscreened intense light sources are altogether unsuitable.   The Pointolite lamp is sufficiently intense to identify the most delicate spirochæta—in fact, even with that it is often advisable to somewhat modify the intensity of the light by means of an absorption screen.   An aplanatic condensing system should be placed in front of the illuminant in the manner already described, except that in the case of a spherical surface dark-ground illuminator (Fig. 33, p. 27), which is the type now usually employed, it is advisable to project upon the surface of the mirror a rough image of the illuminant itself by somewhat lengthening the distance between the illuminant and the illuminant condenser, thus feeding the dark-ground illuminator with a slightly convergent beam.   The accuracy of dark-ground work is almost entirely dependent on perfect centration, the dark-ground illuminator itself therefore, or the sub-stage carrying it, must be

fitted with centring screws. With a light source of moderate
intensity, set up as it should be, and used in a room which is not
too well lighted, there is no difficulty in doing diagnostic work
of this description.

The setting up of the apparatus may be easily and quickly
effected in the following manner :

The light is placed about 10 inches away from the mirror of
the microscope, and a condensing lens, as already described, so
arranged that the image of the radiant is on the centre of the
mirror.   All the optical equipment—*i.e.*, the sub-stage condenser
and the objective—should be removed from the microscope, only
a low-power ocular remaining on it.   If the light is now de-
flected centrally up the tube, a point will be reached where, on
observing the ocular from several inches above its eye-lens, a
perfectly evenly illuminated disc of light will be seen.   Alter-
natively, a piece of paper may be placed 3 or 4 inches above the
ocular, and the reflected image from the paper surface is observed.
A slight deflection of the mirror to one side or the other will
usually quite easily result in an even disc of light being obtained.
This having been effected, under no circumstances should the
light, condensing lens, or the mirror be moved, or the micro-
scope be altered in its relation to the lighting system.

The dark-ground illuminator is now fitted into the sub-stage
and then racked down, the preparation is placed on the stage,
after applying immersion oil to the underside of the slip and
the top surface of the illuminator, which should then be slowly
raised until these oil drops merge into one another.   The
illuminator should be sufficiently raised for the oil layer to
be spread out and completely cover the top surface of the
latter.   This having been effected, a medium-power objective
should be placed on the body tube of the microscope and the
object itself brought into view.   The illuminator should then
be carefully racked up or down until a circular area is illu-
minated occupying a comparatively small part of the field of
view.   This circular area should be as small as possible, and
should be perfectly uniform.   It should further be noted that
the image of the radiant which is now seen should be quite
even on all sides—*i.e.*, there should be no tendency for any
diffusion on one side or the other of this image.   If there is any

such inequality in the appearance of the image, it indicates that the centration of the light has not been carried out properly, and the process must be commenced again.

The high-power immersion objective with which the observations are finally to be made is now placed in position, the lowest power ocular available still remaining in position. On the image now being brought into view, it will probably be found that the illuminated area is not in the centre of the field. This must be brought to the centre by means of the sub-stage centring screws, and this is the only adjustment that is permissible in obtaining an area illuminated as it should be.

A high-power ocular may now be substituted for the lower one to suit the particular work that is in hand. It is usual to employ a $\frac{1}{12}$ oil immersion objective with a funnel stop placed in its back to reduce its aperture for diagnostic work of this kind. It is fairly satisfactory under those conditions, but the recently introduced oil immersion objectives of longer focus—e.g., $\frac{1}{7}$ or $\frac{1}{8}$ inch, having a numerical aperture not exceeding 0·95—will be found much more satisfactory.

If the work is being carried out in a room that is well lighted with daylight, then it is at least advisable that the worker should place his microscope so that his back is to the window—that is, when dark-ground work is being conducted. Alternatively, a blind should be drawn so that the light is reduced. It must be realized that when a bright image is to be seen on a dark ground, it is essential that any extraneous light sources should be modified as far as practicable. The less light there is in the room in which the observations are made, the less intense need the illumination be to observe any given structure or organism. In cases where a very intense light source is used, particularly in the examination of such fluids as blood-serum, the number of granules visible is often so great as to obscure the main image. For this reason, therefore, if for no other, the intensity of the light source should be as much modified as possible.

Ultra-Violet Illumination.

The branch of medical work that calls for the use of the highest powers is bacteriology. Most micro-organisms require to be stained to show satisfactorily their form and structure.

Such staining reactions, from their very nature, often cause changes in the character of organisms, so that a control method in which they can be observed in the living state is desirable. There are two methods available for effecting this—one by dark-ground illumination, as already described, the other by means of ultra-violet light. The former is quite simple in use, and fulfils its purpose if carried out in such a manner as to produce a correct image. The latter is more difficult, requires considerable technical skill, and only gives a photographic result. The apparatus needed is also more complex and is expensive, yet the results obtained are so illuminating that there is no doubt of their value in researches where minute detail has to be resolved. For comparison Plate II. shows two organisms which have been photographed from stained preparations in Figs. 60 and 63, in the living state illuminated by dark-ground in Figs. 61 and 64, also living and illuminated by direct ultra-violet light in Figs. 62 and 65. The relative value of the different images obtained may be inferred from the results.
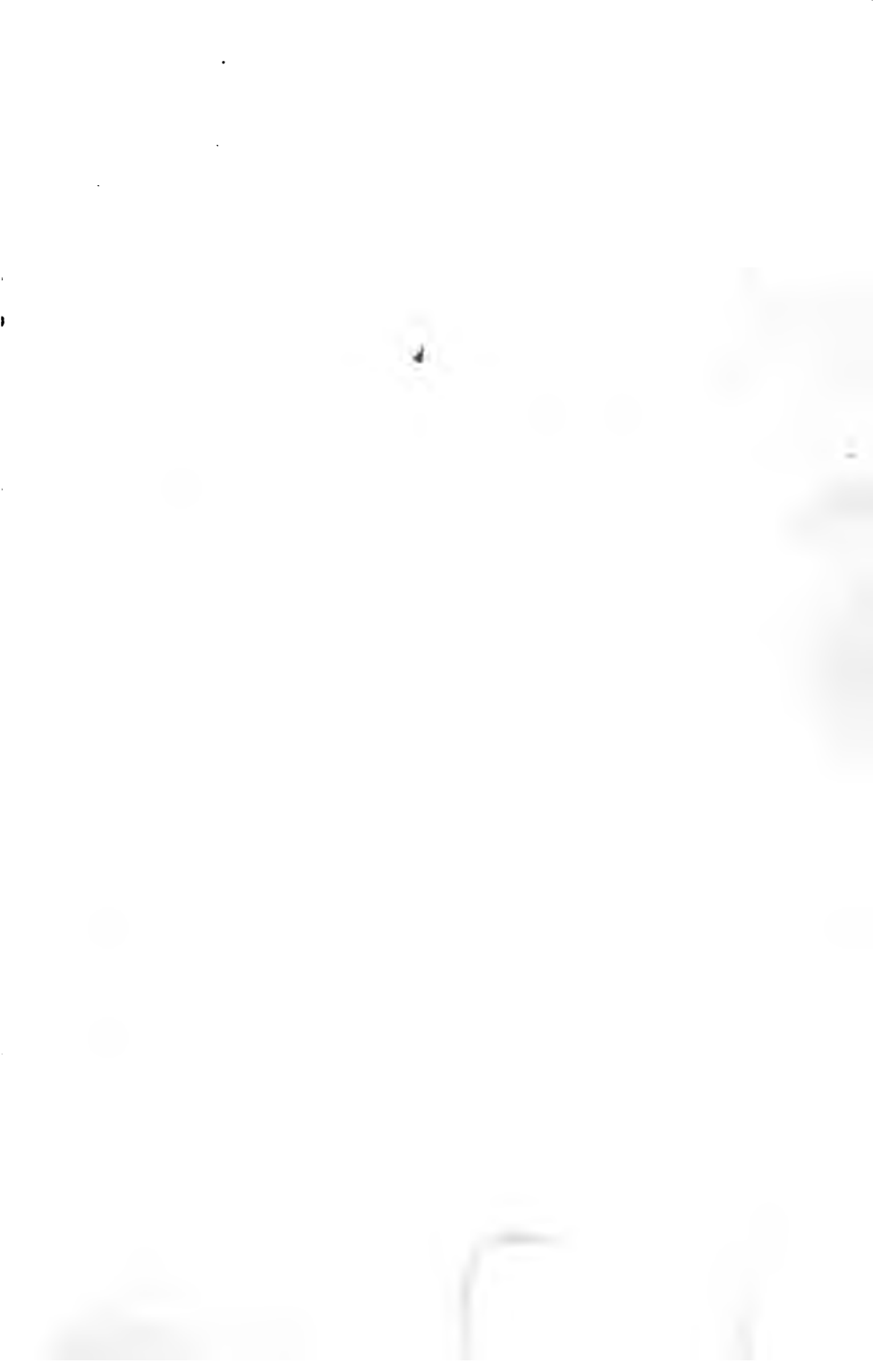
PLATE III.
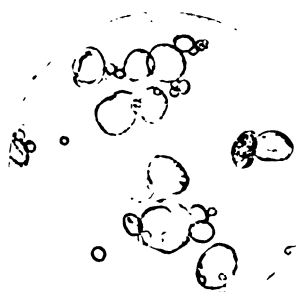
Fig. 66   Starch of Wheat.

Fig. 67.—Starch of Rye.

Fig. 68.—Starch of Barley.

Fig. 69.—Sewage Fungus.

Fig. 70.—Penicillium glaucum.

Fig. 71.—Bacillus anthracis.

THE MICROSCOPE IN MEDICINE—PUBLIC HEALTH

By W. E. COOKE, M.D., F.R.C.P.E., D.P.H., F.R.M.S.

THE study of state medicine embraces parts of many sciences—geology, bacteriology, entomology, etc.—in all of which microscopy plays an essential part, so that it is imperative for anyone taking up that branch of medicine first of all to become thoroughly acquainted with the uses and abuses of the microscope.

The early history of public health is so staged in the dim and distant past that it is difficult to exactly define the period when microscopy first contributed to, or was applied to, the elucidation of its many problems.

Perhaps pride of place may be given to the observations of Van Leeuwenhoek on vinegar about 1780. He described *Anguillula aceti*, and proved to his own satisfaction that the sharp taste of vinegar was not due to the pointed tails of the little nematodes, but to some substance which was neutralized by crushed crabs' eyes. He thus showed that *A. aceti* was a contamination, and not an essential part of vinegar.

In 1849 Pollender noticed a rod-shaped organism in blood of cattle dead of anthrax. The association of the bacillus with the disease recognized by Davaine in 1863 (Plate III., Fig. 71).

From these beginnings the microscope has played such an important rôle that it is difficult to-day to find any part of the Public Health Acts, by-laws, regulations, etc., that is not based upon microscopical observation, or which has not been confirmed by microscopy.

Its place in modern state medicine may be imagined from the following summary of its uses:

1. The examination of air, soil, and water.

2. The detection of adulterations, parasites, and pathogenic organisms in food. ɪ

3. The utilization of micro-organisms for the disposal of sewage.

4. The diagnosis of infectious and parasitic diseases.

5. The investigation and control of the carriers of disease : human, animal, and entomological.

6. Research.

A very brief account will enable us to realize what public health in Britain owes to the microscope, and the vast amount of work still remaining to be accomplished.

1. The Examination of Air, Soil, and Water.

Air.—Air contains particles of soot; débris from the bodies of man and animals (hair, epithelial scales, etc.), from street traffic and industrial operations; the spores and pollen of plants ; fibres of wool, silk, and cotton ; mineral particles and micro-organisms—moulds, yeasts, the organisms of infectious diseases, the tubercle bacillus, sarcinæ, etc.

Pure mountain air contains about 2,000 particles per cubic inch. The number increases as the populous districts are reached, until in overcrowded slums more than 3,000,000 may be counted, a large proportion of which are micro-organisms.

The air of factories and workshops contains débris from the manufacturing processes carried on, such as cotton fibre, woollen fibre, dust, mineral particles, etc., excess of which leads to pulmonary affections, chronic laryngitis, chronic bronchitis, anthracosis, silicosis, and pulmonary tuberculosis. In some processes— e.g., wool-sorting and hair-sorting—the air may contain anthrax spores.

The microscope has led to better ventilation generally, and the framing of the regulations which ensure freedom from risk, and the much-improved conditions under which industrial workers find themselves to-day.

Our knowledge of the aerial transmission of disease is very limited, and much remains to be investigated.

Soil.—In addition to the non-pathogenic organisms found in soil, *B. tetanus*, *B. anthracis*, the bacillus of malignant œdema, *B. typhosus*, *B. coli*, *B. enteritidis sporogenes*, and *B. diphtheriæ*, have all been demonstrated, or cultivated, in the upper layers

of the soil. *Ankylostomum duodenale* and *Ascaris lumbricoides* may be transmitted by soil.

The advantage of the modern practice of thorough drainage of the soil and the rendering impervious of the house site gains confirmation from the above observations.

Water.—Chemical evidence as to the character of a water is presumptive only; the microscope is necessary to furnish the positive evidence as to its purity or otherwise.

Suspended matter, such as sand, chalk, clay, cotton and linen fibres, fragments of leaves and other vegetable tissues, fragments of insects, hair, and wool, may be found, also living organisms— Rhizopoda, Infusoria, Hydrozoa, Insecta, Fungi, Algæ, Diatomaceæ, etc. — which, harmless in themselves, indicate the presence of organic matter for their sustenance.

The ova of *Oxyuris vermicularis* (Plate IV., Fig. 74), *Ascaris lumbricoides*, and *Trichuris trichiura* are said to be transmitted by water.

BACTERIA IN WATER.—These may be grouped into three classes: the ordinary water bacteria, sewage bacteria, and pathogenic bacteria.

The ordinary water bacteria comprise chromogenic and fluorescent organisms, and organisms of the air and soil.

The sewage bacteria include the *B. coli* groups, *B. proteus* group, *B. enteritidis sporogenes*, streptococci, and staphylococci. Found in water, they indicate human fæcal contamination, and therefore possibilities of extensive outbreaks of typhoid fever or cholera, and condemn the water-supply at once.

The chief pathogenic bacteria found in water are the typhoid bacillus and the spirillum of cholera.

The epidemics of typhoid fever that occurred in the last century at Over Darwen, Worthing, Maidstone, and at Lincoln in this century, and the outbreak of cholera at Hamburg, would probably not have occurred if strict microscopical supervision of the water-supplies had been exercised.

2. The Detection of Adulterations, Parasites, and Pathogenic Organisms in Food.

Flour (Plate III., Fig. 66) is adulterated with foreign starches —potato and rice—and alum and calcium sulphate sometimes

added. Fungi and vibriones and *Acarus farinæ* can be detected by the microscope.

Coffee is adulterated with chicory, roasted and ground peas, beans, bran, rye, barley, and acorns.

Flour, maize, and potato starches and clay are found added to mustard, while occasionally tea contains added mineral matter, gypsum, etc. Barley is associated with actinomyces. The Tyroglyphidæ, which infect flour, cheese (Plate IV., Fig. 76), dried fruits, etc., and the Glyciphagi found in sugar, are easily detected by the microscope.

Milk.—This is an essential article of diet, especially for infants and young children. Contaminated, it has been responsible for many epidemics of scarlet fever, typhoid fever, cholera, diphtheria, sore throat, etc., is a constant distributor of tuberculosis, and causes an enormous number of deaths annually from infantile diarrhœa. In addition to the foregoing, anthrax bacilli, *B. dysenteriæ*, and the paratyphoid organisms, may be spread by milk.

The microscope is necessary to check milk-supplies, and gives indications of disease in the cow, the cleanliness or otherwise of the processes of production, storage, and distribution of the milk, and the presence of pathogenic organisms.

Suppurative disease of the udder is detected by an increase in the number of milk leucocytes, and lack of cleanliness in the production by the presence of hair, scales, and straw in the sediment, and by the number of bacteria per cubic centimetre. The number varies from 5,000 to 10,000 in Grade A milk to 2,000,000 to 3,000,000 per c.c. in badly produced and stored milk.

The pathogenic organisms have already been mentioned. It is obvious that more rigid and constant bacteriological control should be exercised over milk-supplies.

Fish.—Epidemics of typhoid fever have been caused by oysters, cockles, and mussels taken from beds contaminated by sewage. *B. coli* and *B. enteritidis sporogenes*, certain evidence of sewage pollution, can be constantly demonstrated in shell-fish. The plerocercoid stage of *Dibothriocephalus latus* is passed in fish, and thus transmitted to man.

Meat.—In addition to tuberculosis, glanders, anthrax, and

PLATE IV.

FIG. 72.—HEAD OF CYSTICERCUS.

FIG. 73.—TRICHINA SPIRALIS.

FIG. 74.—OXYURIS VERMICULARIS.
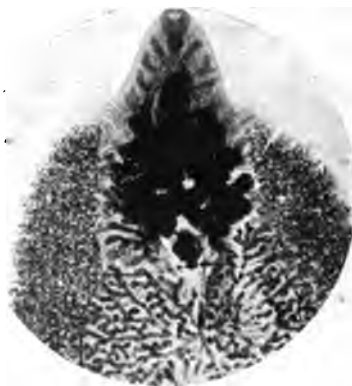
FIG. 75.—LIVER FLUKE OF SHEEP.

FIG. 76.—CHEESE MITES.

FIG. 77.—SEGMENT OF TÆNIA SOLIUM.

actinomycosis, the microscope is necessary to detect moulds, penicillium (Plate III., Fig. 70), mucor, and cladosporium in stored meat, and *B. botulinus*, *B. actrych*, *B. suipestifer*, *B. enteritidis*, *B. paratyphosus*, and certain putrefactive bacteria which have been associated with outbreaks of food-poisoning.

Meat is also responsible for the transmission of certain parasites, the larval stages of which are detected by the microscope. *Cysticercus cellulosæ*, *Cysticercus bovis*, and *Trichinella spiralis* are examples (see Figs. 72 and 73, Plate IV.).

3. The Utilization of Micro-Organisms for the Disposal of Sewage.

This method of sewage disposal is practised extensively. Denitrification organisms, many of which are anaerobes, break down the compounds of ammonia and nitrogen in the sewage, yielding free nitrogen, water, ammonia, carbon dioxide, and a small quantity of nitrites. These bodies are oxidized by the nitrifying organisms, first into nitrites by the nitroso-bacteria, and then into nitrates by the nitric organisms—nitro-bacteria. The microscope is necessary in the identification of the organisms used, and to control the processes of purification (Plate III., Fig. 69).

4. The Diagnosis of Infectious and Parasitic Diseases.

As the causes of disease become more known, laboratory methods will be increasingly called for.

Microscopical diagnosis is utilized in :

(*a*) Bacterial diseases: Typhoid and paratyphoid infections, diphtheria, erysipelas, pneumonia, cerebro-spinal fever, dysentery, cholera, plague, tetanus, glanders, anthrax, tuberculosis, and gonorrhœa, to mention a few.

(*b*) Mycotic infections: Actinomycosis, sporotrichosis, nocardiosis, mycetoma, aspergillosis, etc.

(*c*) Protozoan infections: Amœbiasis, coccidiosis, malaria, relapsing fever, syphilis, and the diseases due to *Trichomonas hominis*, *Lamblia intestinalis*, and *Balentidium coli*.

(*d*) Diseases due to Trematodes, Cestodes, and Nematodes : *Fasciola hepatica* (Plate IV., Fig. 75). *Tænia solium* (Fig. 77),

T. saginata, Dipylidium caninum, Dibothriocephalus latus, the cysticercus stage of *T. solium* and *T. saginata,* the larval stage of *T. echinococcus,* and the nematode infections—*Ascaris lumbricoides, Oxyuris vermicularis* (Fig. 74), trichiniasis, uncinariasis, and *Trichuris trichiura.*

(*e*) Infection by the Arachnida : In England the only common infection is that of *Sarcoptes scabiei.*

(*f*) Parasitic insects : Pediculi, *Cimex lectularius* and *Pulex irritans,* and myiasis, or infection with the larvæ of Diptera.

The student of public health should remember that he may be called upon to deal with imported tropical diseases, as well as those usually met with in Britain.

5. The Investigation and Control of the Carriers of Disease : Human, Animal, and Entomological.

This is one of the most important problems of state medicine. The common diseases in which the human carrier can be diagnosed and controlled are typhoid and the paratyphoid fevers, dysentery (bacillary and amœbic), diphtheria, and cerebrospinal fever. In the course of time all the communicable diseases will doubtless be controlled and epidemics cut short.

The spread of disease through the agency of flies, fleas, bugs, lice, and other insects, is a fruitful field for investigation, and further research may reveal how vast an influence insects have on our public health.

The accompanying tables (pp. 109 and 110) will give some idea of the scope of investigation.

The spread of infection by direct contagion by the common house-fly, typhoid and paratyphoid fevers, cholera, dysentery, etc.; the association of *Stomoxys calcitrans* with anterior poliomyelitis, and *Xenopsylla cheopsis* with plague, are well known.

6. Research.

Lastly, we come to the use of microscopy in state medicine research. About the aerial transmission of disease we know little, and food requires much further investigation.

The question of the domestic animals in relation to the spread of infection has scarcely been touched. The causative organism

PROTOZOAL DISEASES.

Parasite.	Definitive Host.	Intermediate Host.	Important Reservoir of Virus.	Transmission and Pathogenicity.
Entamoeba histolytica	Man	Not required	Man, carrier stage (fæces)	Cysts in food and water. Flies act as carriers. Ingestive. Dysentery.
Balantidium coli ...	Man	Not required	Pig	Ditto.
Lamblia intestinalis ...	Man	?	?	Ditto.
Plasmodium, malariæ, vivax and falciparum	Mosquito (Anopheline species)	Man with schizonts in blood	Man with gametes in blood	Cyclical development in mosquito. Inoculative. Malaria.
Spironema recurrentis	Louse (P. vestamenti)	Man	Man (blood)	Cyclical development in louse. Bite puncture contaminated by crushed louse. Relapsing fever.
Leptospira icterohæmorrhagica	Man and rat	Not required	Rat	Common infection of rats. Present in blood and urine. Ingestive. Weil's disease.
Treponema pallidum ..	Man	Not required	Man	Venereal. Syphilis.
Rickettsia prowazeki....	? Man	Louse	Man	? Inoculative. ? Contaminative. Typhus fever.
Neuroryctes hydrophobiæ	? Man, wolf, dog, etc.	?	Fox, dog, wolf	Inoculative. Hydrophobia.
Cytoryctes vacciniæ ...	Man and ox	Not required	Ox	Inoculative. Vaccinia.
Cytoryctes variolæ ...	Man	?	Man	Smallpox.
Cytoryctes scarlatinæ	? Man	?	Man	? Scarlet fever.

HELMINTHIC DISEASES.

Parasite.	Definitive Host.	Intermediate Host.	Important Reservoir of Virus.	Transmission and Pathogenicity.
Fasciola hepatica ...	Man and sheep	Snail (Limnæa)	Sheep	Cercaria encysted on weeds. Ingestive. Human liver fluke disease.
Ankylostoma duodenale	Man	Not required	Man (fæces)	Larvæ penetrates skin. Ankylostomiasis.
Trichinella spiralis ...	Man, rat, and hog	Hog, man, and rat	Hog (muscle)	Encysted larvæ in raw or insufficiently cooked pork. Ingestive. Trichinosis.
Ascaris lumbricoides ...	Man	Rats	Hog	Larvæ on food. Ingestive.
Dibothriocephalus latus	Man	Cyclops, fish	Dogs, cats	Plerocercoid in muscles. Digestive.
Dipylidium caninum ...	Man	Dog louse or flea, cat louse or flea	Dogs, cats	Cysticercoid from dogs and cats. Ingestive.
Tænia solium ...	Man	Hogs	Man (fæces)	Cysticerci in muscles. Ingestive.
Tænia saginata ...	Man	Cattle	Man (fæces)	Ditto.
Tænia echinococcus ...	Dog, jackal	Man, sheep	Dog, jackal	Dogs infected at abattoir. Ingestive. Hydatid disease.

has not been found in a large number of epidemic diseases. The common cold has baffled investigators up to the present. (Foster's recent work would point to the disease being due to a filterable virus which can be seen by the immersion paraboloid.)

The nature of the filterable virus found in several diseases requires investigation, and the causative organisms of encephalitis lethargica, mumps, measles, rubella, smallpox, scarlet fever, etc., isolating.

Sufficient has been said about the use of the microscope in state medicine to indicate its incalculable importance, not only in that subject, but also in general medicine; yet in many of our medical schools microscopy is not taught.

THE MICROSCOPE IN MEDICINE—TROPICAL DISEASES

By AUBREY H. DREW, D.Sc., F.R.M.S.

(Imperial Cancer Research Fund).

THE microscope has revolutionized our ideas in medicine generally. Diseases for many years completely misunderstood, have been tracked down by its means, and in many cases cures have followed the discovery of the real exciting cause. In no department of medical science, however, has the microscope become more important than in that of tropical diseases. We may call to mind the discovery of the malaria parasites, the bilharzia worm inhabiting the urinary tract, the trypanosomes, the cause of sleeping sickness, and other distressing diseases of man and beast. Without the microscope these diseases must have been for ever beyond the power of man to cope with. Let us take two specific instances as illustrations of the use of the microscope in tracking diseases of this type. We will consider the cases of malaria and sleeping sickness. We have in malaria—or, as it was termed in former days, ague—a disease which is marked by attacks of sharp fever which recur at very definite intervals. The disease was thought to be associated with marshes, as it usually occurred in marshy districts and where much stagnant water accumulated, and drinking marsh water and breathing marsh air, especially at night, was considered one at least of the ways in which malaria was contracted. Microscopic examination of the blood of patients suffering from malaria showed that many of the red blood-corpuscles contained peculiar bodies, which did not occur in normal blood, and which the microscope soon showed went through a definite life cycle. It will be instructive for us to consider this cycle in an elementary manner. If blood be examined in the early

stages before the fever in malaria, we find situated in the red
cells small refractile amœboid bodies which gradually increase
in size. Staining shows that they consist of nucleus or
chromatin, and cytoplasm. As the stage of fever approaches
these bodies take a rosette-like appearance. The chromatin
becomes distributed in granules over the body, and cytoplasm
accumulates round. Finally, the red cell ruptures, and these
little bodies are liberated into the blood-stream, where they
again attack red cells and the process is repeated. It should be
noted that the fever occurs just at the time, or very shortly
after, the rosette-like body is liberated and breaks up into its
little amœbæ. As the disease progresses, we shall sooner or
later find in the blood certain other bodies which are directly
derived from the amœboid forms already described. These are
the sexual forms destined to carry on the life of the parasite
outside the host. These forms are known as gametes, and
show two distinct types. One, which contains more pigment, has
less chromatin and stains more deeply, and is the macro-
gametocyte, or female phase. The other has less pigment,
much more chromatin, and stains much lighter and often in a
different manner, and is the microgametocyte, or male. These
forms cannot undergo any further change in the blood, but
when such blood is ingested by the mosquito a very remarkable
series of changes occur. Roughly speaking, the microgametocyte
gives off numerous thread-like bodies which swim with a lashing
motion. These are the microgametes, corresponding to the
spermatozoa in higher animals. One of these motile bodies
fuses with a female form. The fertilized zygote is termed an
ookinete, and it bores its way through the stomach of the
mosquito and comes to rest in the adjacent tissues. Here it
gradually enlarges and breaks up into hundreds of minute sickle-
shaped bodies. These are then set free by rupture of the
zygote, and make their way to the salivary glands. The
mosquito is now infective, and on feeding introduces some of
these falciform bodies into the circulation of the person
she feeds on. They then become amœboid, and enter the
red cells, and the original cycle is again started. It should be
particularly noted that here we have an alternation of genera-
tions, and this phenomenon is very common amongst such

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parasites.   In the case of sleeping sickness, microscopic examination of the blood shows that it contains numbers of actively motile flagellates termed trypanosomes.   A trypanosome consists of an elongated body, one side of which is thrown into wavy folds called an undulating membrane.   Down the free side of this undulating membrane the flagellum is attached, and is continued at the end into a free waving filament (Fig. B, Frontispiece). Two nuclei are present—a large one termed the trophonucleus, and a very much smaller one, the kinetonucleus.   The trypanosomes multiply in the blood-stream by a process of division.   The disease is transmitted to man by the tsetse fly.   When blood containing trypanosomes is ingested by the tsetse fly, the flagellates undergo a series of changes which finally produce trypanosomes, which are again inoculated into the blood when the fly feeds.   All these facts are due to careful microscopic study, and it will be readily understood that for much of this work, involving as it does examination of very minute forms, a first-class microscopic outfit is necessary.   When travelling in the bush or jungle, a portable microscope is exceedingly convenient and often a necessity.   Fig. 110, p. 218, shows a good pattern of this type, with which the highest powers can be used. For detailed examination in the laboratory the writer prefers one of the larger stands such as those figured in Chapter VI.   Four objectives will practically cover the whole field of work, the most useful being a 2 or 3 inch, a $\frac{1}{2}$ inch, a $\frac{1}{6}$ inch, and a $\frac{1}{12}$ inch oil immersion, which should preferably be an apochromat. The 3 inch will often be used for detailed examination of insects such as mosquitoes, flies, etc.   For minute dissection of insects so often necessary a dissecting microscope is essential.   It will be convenient for us to consider next some of the methods to be employed in the examination of the commoner parasites likely to be met with.

Examination of Blood for Malaria.—The best method for searching for the malaria parasite is to spread thin films of the blood on slides.   This is best done by placing a drop of the blood on one end of a slide and applying a second slide to it at an angle of about 45°.   The second slide is then drawn steadily along, and the blood spreads out into a thin layer.   Some observers find much difficulty in making films in this manner.   Where

such is the case, the film may be made by the cigarette-paper method. A drop of blood is placed on the end of a slide, and a narrow strip of cigarette-paper is applied to it. The blood spreads out under the end of the paper, which is then drawn across the slide. A successful film should show the cells evenly spread in a single layer. Whichever method of spreading is adopted, the slides should be allowed to dry in air, and then be placed in absolute methyl alcohol to fix. They should remain in this fluid for at least an hour. If methyl alcohol is not to hand, equal volumes of alcohol and ether may be used.

STAINING.—Such films may be stained in a ½ per cent. solution of eosin in distilled water for thirty seconds, washed, and then stained in a saturated watery solution of methylene blue for about thirty seconds. The films are then washed, dried, and mounted in balsam. When this method is successful the red blood-cells should be stained pink, and the nuclei of the leucocytes and the malarial parasite should be blue. The easiest and best method for staining such films is the Giemsa method. Giemsa's solution may be bought of dealers such as Messrs. Baird and Tatlock, Hatton Garden, or may be prepared from the eosin-azur Soloids sold by Messrs. Burroughs Wellcome and Co. A Soloid is dissolved in 5 c.c. of a mixture of equal parts pure absolute methyl alcohol and glycerine. For use, one drop of the eosin-azur solution is added to 1 c.c. pure neutral distilled water, and the film is stained for an hour in this solution. It is then washed under the tap, dried between filter-paper, and may then be examined either by being mounted in balsam, or may be examined direct with the oil immersion lens without a cover-glass. The usual cause of failure in this operation is the use of water which is acid in reaction. The ordinary laboratory distilled water will not do, as it is always acid by reason of the CO_2 which it contains. The best method of preparing such water for use is to place a litre or so in a large clean glass flask and to boil vigorously for ten minutes. This will remove the whole of the CO_2, and such water may be used for Giemsa staining for a day or two. In a successful preparation the red cells should be stained yellowish-pink, the nuclei of the leucocytes a purple, whilst the cytoplasm of the lymphocytes and the malarial parasites will be

stained blue. The parasites show a purple staining chromatin mass corresponding to a nucleus.

Some observers prefer Leishman's stain, as it is very rapid. The solution is best purchased ready made. Films should be prepared as already described, but they should only be dried, and not fixed. About twenty drops of the undiluted stain are poured on and allowed to act for thirty seconds by the watch. An equal quantity of distilled water is then added, and the diluted stain allowed to act for about five minutes. The film is then washed in distilled water, dried and mounted. The red blood-cells are pink, nuclei of leucocytes red-purple, and the parasites are blue with reddish nuclei. The same methods may be employed for the staining of trypanosomes and other blood parasites.

Examination of the Fæces.—In tropical dysentery it is necessary to examine the fæces for the dysentery amœba, *Entamœba histolytica* or its cysts, or it may be required to examine for the presence of other protozoa. For the examination of the fresh fæces, a small quantity may be removed and mixed with normal salt solution to form a thin emulsion, and a drop or two of this examined on a warm stage. For fixed and stained preparations the method of Woodcock may be used with advantage. A loopful of fæces is taken up by means of a platinum loop, and is mixed with a drop or two of 0·5 per cent. salt solution; a thin smear of this diluted fæcal emulsion is spread on a slide, and the preparation is at once, without drying, placed in a tube containing a pad of wool saturated with 4 per cent. osmic acid plus one drop of glacial acetic acid. The slide is left to fix in the osmic vapour for about ten or twelve seconds, and is then removed and allowed to dry in the air. It is then placed in absolute alcohol for a quarter of an hour, washed under the tap, and stained with Giemsa's solution; one drop to 1 c.c. Amœbæ cysts may be readily identified if a loopful of diluted fæces is mixed on the slide with a drop or two of Gram's iodine solution.

Mosquito Dissection.—It is often necessary, in studying the insect cycle of various protozoa, to dissect the insect hosts, such as the mosquito or tsetse fly, so that the methods adopted in the case of, say, the dissection of the mosquito will serve as a guide to other insect dissections. The insect may be

killed by means of chloroform vapour. The mosquito should have the legs and wings pulled off with fine forceps, and the body is then to be transferred to some 0·85 per cent. salt solution. The dissection may be carried out by aid of needles and the dissecting microscope, but as no great magnification is necessary the best form of magnifying lens to use is a binocular type, fitting on like ordinary spectacles, and leaving the hands quite free. The anterior end of the thorax of the insect is fixed with pressure from a dissecting needle, and with the other needle the chitinous joint between the sixth and seventh segments of the abdomen is torn slightly. If the thorax of the insect is now held with one needle, whilst the last segments are pulled away in the opposite direction with the other, the alimentary canal will be drawn out and float in the salt solution. The stomach should be examined for the zygotes of the malarial parasite. These often show up as protuberances on its outer side. The salivary glands should be examined for sporozoites, or the form of parasite inoculated by the bite of the mosquito. The best way to dissect out the salivary glands is to press the anterior part of the thorax and then gently draw away the head; the glands come away with the head segment. The easiest method of searching for parasites is to cover the glands with a cover-glass and to hunt over with a $\frac{1}{6}$ inch dry lens for sickle-shaped bodies. Should these be observed, make a smear by crushing the glands on a slide, dry in air, and stain with Leishman. Minchin's method for staining flagellates from the gut of insects will be found of great use where such forms are being studied. The method is best applied as follows :

1. Fix the wet films from the gut in saturated watery per-chloride of mercury two parts, alcohol one part.

2. Wash in 50 per cent. alcohol fifteen minutes.

3. Wash in water about fifteen minutes.

4. Wash again in water to which is added a few drops of Gram's iodine solution.

5. Wash in 1 per cent. hyposulphite of sodium.

6. Wash in running water.

7. Stain in Giemsa for twelve hours (one drop to 1 c.c.).

8. Differentiate in three strengths of acetone-xylol for about five minutes in each. The strengths are: No. 1, acetone 95

per cent., xylol 5 per cent. No. 2, acetone 70 per cent., xylol 30 per cent. No. 3, acetone 50 per cent., xylol 50 per cent.

9. Transfer to pure xylol and mount in balsam.

Spirochætes.—Several diseases are now known to be due to infection with spirochætes, many of which are conveyed by the bite of ticks. For their detection in blood we may either examine it fresh by means of the dark-ground illuminator, or we may fix in methyl alcohol and stain for twenty-four hours in Giemsa (one drop to 1 c.c.). Gunther's method will at times be found useful.

1. Prepare films, and dry by gently heating high over a bunsen flame.

2. Place in 5 per cent. acetic acid for thirty seconds.

3. Expose to ammonia vapour for a few seconds.

4. Wash in distilled water.

5. Stain in Ehrlich's aniline violet for ten to fifteen minutes.

6. Mount and examine with the $\frac{1}{12}$ inch oil lens.

For the examination of tissues for *Treponema pallida* and other spirochætes we may use a modification of Levaditi's method, as follows :

1. Cut the tissues into pieces about 1 to 2 mm. and fix in 10 per cent. formol in 0·85 salt solution for twenty-four hours.

2. Harden in absolute alcohol for twenty-four hours.

3. Transfer to distilled water, and wash till the pieces sink to the bottom.

4. Place in—

1 per cent. aqueous silver nitrate				...	90 c.c.
Pyridine	...	...	...	...	10 c.c.

Leave in this solution for three hours at room temperature, and then place in water-bath at 50° C. for three to five hours.

5. Wash thoroughly in distilled water.

6. Place in—

4 per cent. aqueous pyrogallol	...		...		90 c.c.
Pure acetone	...	...	...	...	10 c.c.
Pyridine	...	...	...	...	17 c.c.

Leave for four hours at room temperature. It should be noted that this solution must be prepared fresh ; it will not keep.

7. Dehydrate in absolute alcohol, transfer to xylol, embed in paraffin, and cut sections.

8. Stain sections on slide with 2 per cent. aqueous toluidine blue, and differentiate in alcohol.

9. Dehydrate in absolute alcohol, clear in oil of bergamot and xylol.

10. Mount in balsam.

In this method the spirochætes are stained black, the groundwork of cells is blue.

Bacteria.—The examination of material for the presence of various pathogenic bacteria such as the vibrio of cholera, the plague bacillus, and the organism of actinomycosis, will often be necessary. The usual bacteriological technique will be used here, but the following hints may be of service:

EXAMINATION OF FÆCES FOR THE CHOLERA VIBRIO.—One of the small mucous pieces should be selected, spread on a slide fixed by passage through the flame, and stained for five minutes in diluted 1 to 10 carbol-fuchsin.

For examination of the vibrio alive, rub one of the mucous patches in a drop or two of salt solution, place a drop of the emulsion on a cover-glass, and invert over a well-slide. Seal with vaseline, and examine the hanging drop with a $\frac{1}{6}$ inch lens.

PLAGUE: EXAMINATION OF THE PUS FROM THE BUBOES.—The blood or juice from enlarged lymphatic glands may be undertaken in a search for the plague bacillus.

Films should be prepared, fixed in alcohol-ether, and stained with carbol-fuchsin. A film must also be stained by Gram's method, the plague bacillus being Gram-negative. For Gram's method of staining, the modification by Nicolle, perhaps, will be found the most useful. It is as follows:

1. Stain in carbol gentian violet five minutes.

2. Without washing, transfer to Lugol's iodine solution as below:

Iodine...	...	...	...	...	1 gramme.
Potassium iodide	...	...	...	2 grammes.
Distilled water	...	...	...	300 c.c.

Leave in this solution for five minutes.

3. Wash in water.

4. Pour on alcohol-acetone (alcohol 10, acetone 1) drop by drop till no more colour comes away.

5. Wash in water and counter-stain in diluted carbol-fuchsin for thirty seconds.

6. Wash in water, dry, and examine with the immersion lens.

All organisms retaining the stain by Gram are stained a blue-black, and the Gram-negative forms are stained red by the counter-stain.

The foregoing notes should enable an observer to understand some of the many problems connected with tropical disease in which the microscope is an indispensable agent, but in few other forms of study is it so necessary to use the microscope properly. It cannot be too well understood that the microscope is not and cannot be a mere magnifying glass. It must be used in an intelligent manner, and in accordance with the laws of microscopical optics; then, and then only, are we getting a picture that can be relied upon at all as being anything like the original object.

This point is of the greatest importance, as faulty images lead only too frequently to wrong deductions. There is probably no other scientific instrument which is placed in the hands of students without any instruction as to its use, and yet the student is generally expected to be able to use a microscope without the slightest teaching as to modern methods of microscopy.

THE MICROSCOPE IN HISTOLOGY

By M. J. COLE.

In publishing methods of preparing, staining, hardening, and mounting the tissues of animals and plants, I have adopted the system employed in my classes for some years past—that is, each separate stage of procedure is arranged in successive lessons, and the whole epitomized on pp. 164-167 at the end of the chapter. A subject such as this cannot be so lucidly described in writing as by demonstration, but it has been my aim to make it as clear as possible, so that, if the instructions are carefully followed and practised, successful permanent work can be performed; but it is only by most scrupulous care and constant practice that any degree of success in this work can be attained.

In books on this and cognate subjects it too often happens that tools, instruments, and routine are prescribed that tend to make work needlessly laborious and expensive, and are in consequence causes of discouragement to the readers. The directions given in the succeeding pages will, it is believed, commend themselves for their directness and simplicity. They are, moreover, thoroughly practical, and are the processes that I have found the most effective after more than forty years' experience as a mounter of microscopic objects.

Lesson I.

HARDENING AND PRESERVING ANIMAL TISSUES AND ORGANS FOR MICROSCOPICAL EXAMINATIONS.

Fresh untreated tissues are unsuited for microscopical purposes, but it is sometimes advisable to observe the appearance of some specimens, such as muscle fibres, tendon, connective tissues, and

nerve fibres, while fresh. When this is desired, the tissue must be examined in certain fluids called 'normal fluids' that will alter its character as little as possible. Those generally used are: (1) Blood-serum; (2) the aqueous humour from a fresh eye; and (3) normal or ¾ per cent. salt solution. The two former are difficult to obtain, but the latter can be made at any time, and it will answer for most purposes. Place a small piece of the tissue on a slide, add a drop or two of salt solution, take two needles fixed in holders and carefully separate the fibres from each other; this process is called teasing. When sufficiently teased, apply a cover-glass and examine. You may now wish to irrigate with some staining reagent. If so, place a few drops of the stain at one edge of the cover-glass, and apply a piece of blotting-paper to the other side. This will absorb the salt solution, and the staining fluid will follow and take its place around the tissue. The slide may then be placed under the microscope, and the action of the reagent observed.

These specimens cannot, as a rule, be kept. For permanent preparations the tissues or organs must be hardened. This is accomplished by subjecting them to the action of certain hardening or fixing solutions. The following are most commonly used:

Absolute Alcohol.—Suitable for stomach, pancreas, and salivary glands. These organs must be perfectly fresh, and they should be cut into small pieces, so that the alcohol may penetrate as quickly as possible.

Change the alcohol every day for the first three days. The hardening is usually complete in a week.

Chromic Acid and Spirit.—Chromic acid ⅙ per cent., watery solution 2 parts, and methylated spirit 1 part. This reagent hardens in about ten days. Then transfer to methylated spirit, which should be changed every day until no colour comes away from the tissues. It is suitable for cartilage, nerve trunks, heart, lips, bloodvessels, trachea, lung, tongue, bladder, ureter, intestines, and œsophagus.

Potassium Bichromate.—Make a 2 per cent. watery solution. This will harden in about three weeks. Then transfer to methylated spirit, and change the spirit every day until no colour comes away from the tissues. It is suitable for muscle, spleen, liver, and kidney.

Ammonium Bichromate.—Make a 2 per cent. watery solution. It hardens in from three to four weeks. Then transfer to methylated spirit, and change every day until no colour comes away from the tissues. It is suitable for spinal cords, medulla, pons Varolii, cerebellum, and cerebrum.

Müller's Fluid.—Bichromate of potash 30 grains, sulphate of soda 15 grains, distilled water 3½ ounces. It hardens in from three to five weeks. Then transfer to methylated spirit, and change every day until no colour comes away from the tissues. Suitable for lymphatic glands, eyeballs, retina, and thymus gland.

Methylated Spirit.—May be used universally if preferred, but it has a tendency to shrink some tissues too much. It hardens in about ten days. Change the spirit every twenty-four hours for the first three days. Suitable for skin, scalp, testicle, penis, prostate gland, vas deferens, epididymis, ovary, uterus, Fallopian tubes, placenta, mammary gland, suprarenal glands, tonsils, and all injected organs.

Decalcifying Solution.—For bones. Make a ⅙ per cent. watery solution of chromic acid, and for every ounce add 5 drops of nitric acid. This fluid will soften the femur of a dog in about three weeks; larger bones will take longer. Change the fluid several times, and test its action by running a needle through the thickest part of the bone. If it goes through easily, the bone is soft enough; if not, continue the softening process a little longer. When soft enough, transfer to water, and soak for an hour or two; then pour off the water and add a 10 per cent. solution of bicarbonate of soda, and soak for twelve hours to remove all trace of acid. Wash again in water, and place in methylated spirit until required. Bones and teeth should always be softened in a large quantity of the decalcifying solution.

For Olfactory Region divide with a saw the head of a freshly killed rabbit or guinea-pig longitudinally, and parallel with the nasal septum. Cut out the septum so as to expose the olfactory region, which is recognized by its brown colour. Dissect out a portion, including some of the turbinated bones. Harden this in Müller's fluid for three or four days. Then transfer to chromic and nitric acid decalcifying solution, and soak until

the bones are quite soft. Wash well in water to remove all trace of acid, and complete the hardening in methylated spirit.

For Cochlea dissect out the internal ear of a freshly killed young guinea-pig, open bulla with bone forceps, when a conical elevation, the cochlea, will be seen. Remove as much of the surrounding bone as possible, and place the cochlea in Müller's fluid for two weeks to harden the delicate nervous tissues. Then transfer to chromic and nitric acid decalcifying solution, and soak until the bone is soft. Place in weak spirit for a day or two, and then transfer to strong methylated spirit.

Corrosive Sublimate.—Tissues may be fixed very quickly in corrosive sublimate. Make a saturated solution in 5 per cent. glacial acetic acid. The specimens should be removed from the solution as soon as they are fixed, directly they become opaque throughout. Then wash in repeated changes of 70 per cent. alcohol, to which a little tincture of iodine has been added. This process will fix tissues in a few minutes.

Picric Acid.—Make a saturated solution in water. This solution will fix small pieces of tissue in a few minutes; larger specimens will require from three to six hours' immersion. Then wash out the picric acid with repeated changes of spirit. Water must not be used, as it is hurtful to the tissues that have been prepared by this method. For the same reasons, during all subsequent changes of treatment, water should be avoided, and the staining should be carried out in alcoholic solutions.

Formaldehyde.—This may be used universally if required. It is sold commercially as ' formal ' in a 40 per cent. solution. This must be reduced by the addition of water to a 2 or 4 per cent. solution. It is specially useful for hardening nervous tissues and for eyes ; the latter are completely hardened in twenty-four hours.

When in great haste, tissues may instantly be fixed in boiling water. Boil some water in a test-tube, then drop in small pieces of the tissue, and boil again for a few seconds. The specimen may then be placed at once in gum and syrup, and when penetrated, freeze, and make the sections. This method should only be used when a section is urgently wanted.

General Directions for Hardening Tissues.

1. Always use fresh tissues.
2. Cut the organs into small pieces with a sharp knife.
3. Never wash a specimen in water; when it is necessary to remove any matter, allow some normal salt solution to flow over the surface of the tissue, or wash in some of the hardening reagent you are going to use.
4. All specimens should be hardened in a large quantity of the reagent; too many pieces should not be put into the bottle, and they should be kept in a cool place.
5. In all cases the hardening process must be completed in spirit.
6. Label the bottles, stating the contents, the hardening fluid used, and when changed. Strict attention to these details is necessary for successful histological preparations, for if the hardening is neglected good sections cannot be made.

LESSON II.

EMBEDDING TISSUES AND SECTION CUTTING.

To Cut Sections with a Razor by Hand.—Take the tissue between the thumb and forefinger of the left hand. Hold the finger horizontally, so that its upper surface may form a rest for the razor to slide on. Take the razor, hold it firmly in the hand, keep the handle in a line with the blade, and draw it through the tissue from heel to tip towards yourself. While cutting, keep the razor well wetted with dilute methylated spirit, and as the sections are cut place them in a saucer of dilute methylated spirit.

Embedding in Paraffin Wax and Lard.—Melt together by the aid of gentle heat four parts of solid paraffin and one part of lard. A quantity of this may be made and kept ready for use at any time. Melt the paraffin mass over a water-bath. Take the specimen and dry it between the folds of a cloth to remove the spirit, so that the paraffin may adhere to its surfaces, place it in a pill-box in the desired position, and pour in enough melted paraffin to cover it, then set aside to cool. When quite cold, break away the pill-box and cut sections from the embedded mass

with a sharp razor. When a number of specimens are embedded, and it is desired to keep them for some time, they should be preserved in a jar of methylated spirit.

To Infiltrate a Tissue with Paraffin.—Dehydrate the specimen in absolute alcohol for several hours, then transfer to chloroform or xylol, in which it must remain until perfectly saturated. When clear, place in a bath of melted paraffin of 45° C. (melting-point), and keep it at this temperature for several hours, so that the paraffin may penetrate to the middle of the tissue. Then remove it from the paraffin and put it into a small pill-box, pour in enough paraffin to fill the box, and as the paraffin cools, add a little more to make up the shrinkage and set aside to cool. When cold, place in water for a few minutes; this will soften the paper, and facilitate the removal of the pill-box. You will now have a cylinder of paraffin with the specimen firmly fixed in its centre, and, if desired, the paraffin may be pared away from the sides until a square block is obtained. The sections may now be made by hand with a razor, or the block can be fixed to a microtome with a little melted paraffin. The sections must be placed in turpentine to remove the paraffin, then in absolute alcohol to remove the turpentine, and, finally, in distilled water to remove the alcohol; they may then be stained. Sometimes it is desirable to stain the tissue in bulk before it is embedded. In this case the sections need only go into turpentine or benzole to wash away the paraffin; they may then be mounted in Canada balsam.
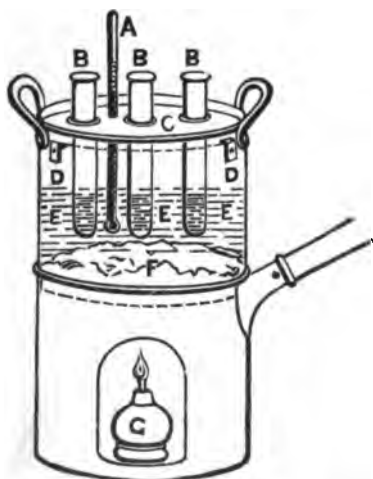

Fig. 78.—Potato-Steamer converted into an Embedding Bath.

A, Thermometer; B, test-tubes; C, disc of tin; D, tin supports; E, water; F, cotton-wool; G, spirit or small paraffin lamp.

The above process requires an embedding bath. This is usually an expensive affair, but one that will answer all ordinary purposes can easily be made.

Get a small potato-steamer, and cut a hole in the lower vessel to admit a spirit or small paraffin lamp. Get a tinsmith to cut out a circular plate of tin to fit into the upper vessel, in which some holes must be cut to take the test-tubes, and to the sides of the vessel four small pieces of tin, bent at right angles, must be soldered to support the tin plate. A piece of tin must also be soldered over the perforated bottom of the vessel, so that it will hold water. When the alterations are complete, place a layer of cotton-wool or a piece of felt on the bottom of the steamer, to protect the test-tubes from breakage ; half-fill with water, add a thermometer, light the lamp, and on the desired temperature being attained, put some paraffin in the test-tubes, place them

FIG. 79.—COLE'S PATTERN MICROTOME.

in the steamer, and when the paraffin has melted add the specimens.

After use dry the apparatus so that rust may not set in. If this is attended to it will last for years.

When a proper embedding bath cannot be obtained, tissues may be infiltrated with paraffin in the following way : Dehydrate the specimen in absolute alcohol ; then place in a quantity of chloroform or benzole, ten or twelve times the bulk of the tissue, until saturated ; add small pieces of paraffin until no more will dissolve, and set aside for several hours. Apply gentle heat to drive off the solvent and melt the paraffin, after which the tissue can be removed and embedded in a pill-box of paraffin of the desired melting-point.

Cole's Microtome and Embedding in Carrot.—When a

number of sections are wanted, or when a complete section of
an organ is desired, a microtome should be used such as the
simple pattern illustrated on p. 127.   Screw the microtome firmly
to the table, and with the brass tube supplied with the micro-
tome punch out a cylinder of carrot to fit into the well of the
microtome.   Cut this in half longitudinally, and scoop out
enough space in one half of the carrot to take the specimen;
then place the other half of the carrot in position, and make sure
that the specimen is held firmly between them, but it must not
be crushed.   Now put the cylinder of carrot and specimen into
the well of the microtome and commence cutting the sections.
A good razor will do, but it is better to use the knife which is
made for use with the microtome.   While cutting, keep the
knife and plate of the microtome well wetted with dilute methy-
lated spirit, and as the sections are cut place them in a saucer
of dilute spirit.   A number of sections may be cut and preserved
in methylated spirit until required.

When a specimen has a very irregular outline, it cannot be
successfully embedded in carrot.   Paraffin should then be used.
Place the tissue in the well of the microtome in the desired
position, pour in enough melted paraffin to cover it, and when
cold cut the sections.

Freezing Microtome.—Cathcart's is the most simple and
cheapest freezing microtome, and it can be obtained from any
optician (Fig. 80).

1. Cut a slice of the specimen about ⅛ inch thick, in the
direction you wish to make the section.

2. Place in water for an hour to remove the alcohol.

3. Transfer to a mixture of gum-water 5 parts, saturated
watery solution of loaf-sugar 3 parts, and allow it to soak in
this for about twelve hours; or, if a few drops of carbolic acid
are added to the mixture, tissues may remain in it for months
without harm.

4. Clamp the microtome to a table, fix the ether spray in its
place, and fill the bottle with ether.   Methylated ether, specific
gravity 720, will do.

5. Put a little gum and syrup on the zinc plate of the
microtome, and place the tissue in it.   Commence working the
bellows, and as soon as all the gum has frozen add some more

and freeze again, and so on until the tissue is completely covered and frozen into a solid mass. This proportion of gum and syrup works well in a temperature of 60° F. ; but when higher, less syrup is required—when lower, more. The syrup is used to prevent freezing too hard, so some judgment must be exercised in the matter.

6. The best instrument for making the sections is the blade of a carpenter's plane. Hold it firmly in the right hand, and work
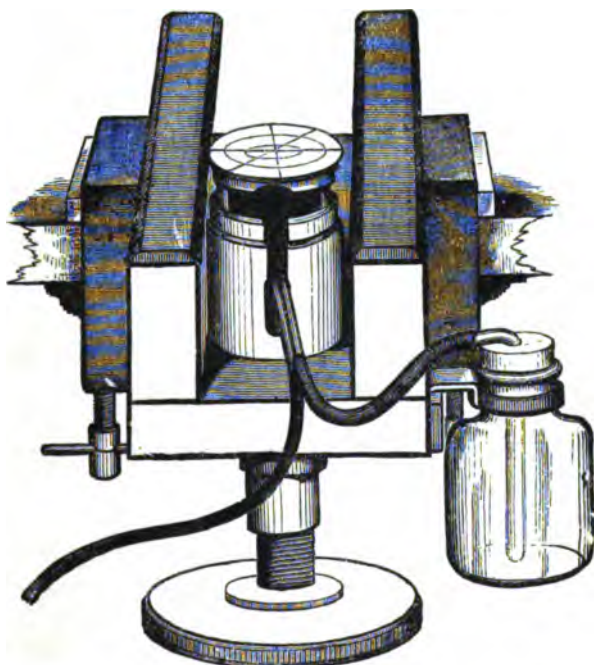
FIG. 80.—CATHCART'S MICROTOME.

the microtome screw under the machine with the left. Plane off the sections as quickly as possible. They should all collect on the plane iron. If they roll up or fly off, the tissue is frozen too hard, or there is not enough syrup in the gum. If the former is the case, allow the mass to thaw a little ; if the latter, add some more syrup to the gum mixture, and soak the tissue again.

When the sections are cut, place them in a saucer of water, which must be changed several times until all trace of gum is

removed. Water that has been boiled and allowed to cool will remove the gum sooner than cold water. When quite free from gum, the sections may be bottled up in methylated spirit until required for staining.

Embedding in Celloidin.—Dissolve Schering's celloidin in equal parts of absolute alcohol and ether until the solution is as thick as glycerine. Divide the solution into 2 parts, to 1 of which add an equal part of absolute alcohol and ether. Dehydrate the specimen in absolute alcohol for several hours, then transfer to the thinner solution of celloidin, and soak until perfectly saturated; place in the thick celloidin for about an hour, or until required. Take a cork and paint over one end a layer of celloidin, and let it dry; this will prevent air bubbles rising from the cork and lodging in the mass. Take the specimen from the celloidin and lay it on the cork, and let it stand for a minute or two, then add some more celloidin until the tissue is completely covered, and set aside, and when the mass has attained such a consistency that on touching it with the finger no impression will remain, place it in 50 per cent. alcohol for an hour or two to complete the hardening, or it may remain there until required. The embedded mass can now be placed between two pieces of carrot, and put into an ordinary microtome, and the sections made with a knife or razor, which must be well wetted with methylated spirit; or the embedded specimen can be removed from the cork, and, after soaking in water, it can be transferred to gum and syrup, and the sections made with a Cathcart freezing microtome. If it is desired to remove the celloidin from the sections, soak them in equal parts of absolute alcohol and ether. When all the celloidin is removed, transfer to distilled water, then into the stain. After staining, wash in distilled water, dehydrate, clear in clove oil, and mount in Canada balsam.

When it is not desirable to remove the celloidin from the sections, they should be stained in borax carmine or hæmatoxylin. The former stains celloidin, but the colour is removed by washing in acidulated alcohol. Hæmatoxylin only stains it slightly. All the aniline dyes stain it deeply; they should not be used.

Tissues are usually stained in bulk before they are infiltrated with celloidin. When so, the sections must be dehydrated in

methylated spirit, cleared in oil of bergamot or origanum, and mounted in Canada balsam.

When desirable, sections infiltrated with celloidin may be mounted in Farrant's medium or glycerine jelly. Wash away all trace of alcohol with water, and mount in either of the above media in the ordinary way.

Celloidin is an excellent medium for infiltrating many specimens of both animal and vegetable subjects. The following are a few of these :

Flower-buds of lily, yucca, evening primrose, poppy, dandelion, and anthers ; worms, leech, flukes, gills, and organs of mussels, heads of frogs, newts, sponges, etc.

For flower-buds proceed as follows : Harden the bud in methylated spirit in the ordinary way. Then take a piece of fine silk or cotton and tie it round the centre of the bud to hold the parts together ; now with a sharp knife cut off each end of the bud so that the celloidin may easily penetrate to the interior. Now place the specimen in equal parts of absolute alcohol and ether for at least twelve hours. Then transfer to the thin solution of celloidin, and soak until completely infiltrated. Remove and place in thick celloidin for about twelve hours. Take out of celloidin on the point of a needle, and hold exposed to the air for a few minutes, to dry the celloidin around the exterior of the bud. When dry, push gently off the needle into some methylated spirit, and soak for at least twelve hours to complete the hardening of celloidin. The specimen may then be embedded in carrot, and the sections may be cut in any ordinary well microtome. Worms must be cut up into pieces of about $\frac{1}{4}$ or $\frac{1}{2}$ inch long ; these are then dehydrated in equal parts of ether and alcohol, infiltrated with and embedded in celloidin, and then treated in exactly the same way as directed for flower-buds.

When a number of celloidin masses are prepared for future use, they must be preserved in a vessel of methylated spirit.

Embedding in Gelatine.—This method is very useful for hairs, cotton, silk, wool, and all such fibres. Take, for example, some human hairs about $\frac{1}{2}$ inch long, and make a bundle of them ; tie them together either with a long hair or with some fine cotton. Place the bundle in warm water and soak for a few minutes. Now make up a strong solution of some clear

transparent gelatine. Cox's is very good—say 1 ounce of gelatine to 6 of water. Transfer the bundle of hair to this, place in a warm water-bath, and soak until the gelatine has penetrated all through the bundle. Remove from gelatine on the point of a needle, and allow the mass to cool; then place in methylated spirit for about twelve hours. The embedded mass may then be placed in a cylinder of carrot and transverse sections cut in the ordinary well microtome. The sections when cut are to be placed in strong spirit to dehydrate; they are then cleared in clove oil and mounted in Canada balsam.

Heads of frogs, newts, and many other specimens may be

Fig. 81.

infiltrated and embedded in gelatine, but they must all be stained in bulk before they are infiltrated, because the sections must not come in contact with water in any form; moreover, if the sections were stained the gelatine would be coloured as well as the tissues.

The Rocking Microtome.—This machine is made by the Cambridge and Paul Instrument Company. It is only used for specimens infiltrated with paraffin, and it is automatic—that is to say, it can be set to cut sections of definite thickness, and every time the handle is pulled a section is cut, and the specimen is moved forward ready for another (Fig. 81).

Infiltrate the tissue with paraffin in the ordinary way in a pill-box, and when the paraffin has set, remove the box and trim the paraffin into a rectangular block. Take care to keep the edges quite parallel, so that they may adhere together, as

the sections are cut and form a riband. The Cambridge and Paul Instrument Company make an apparatus for embedding, called embedding L's.   If these are used, perfectly rectangular blocks are formed ready for fixing to the brass cap at the end of the arm of the microtome, which is filled with paraffin; this should be warmed over a spirit-lamp, and the block containing the specimen is to be pressed against the melted paraffin until it adheres firmly.

<div align="center">Lesson III.</div>

STAINING ANIMAL SECTIONS AND MOUNTING IN CANADA BALSAM.   .

All sections of organs and tissues should be stained with some colouring reagent, so that their structure may be made more apparent.   Certain parts of the tissue have a special affinity for the dyes or stain; they therefore become more deeply tinted, and stand out clearly from the surrounding tissues.

The following staining reagents are the most useful :

Grenacher's Alcoholic Borax Carmine. — Carmine, 3 grammes; borax, 4 grammes; distilled water, 100 c.c.   Dissolve the borax in the water, add the carmine, and apply gentle heat until all is dissolved; then add 100 c.c. of 70 per cent. alcohol, filter, and keep in a stoppered bottle.

Staining Process.—1. Place the section in distilled water to wash away the alcohol, then place a little of the carmine in a watch-glass, and immerse the section for from three to five minutes.

2. Wash the section in methylated spirit.

3. Take of methylated spirit 5 parts, and of hydrochloric acid 1 part, and mix them well together.   A quantity of this acid solution may be made up and kept ready for use at any time.

Immerse the section in the above, and leave it to soak for about five to ten minutes, or, if overstained, until the desired tint is obtained.   Sections of skin and scalp may be left until all colour is removed from the fibrous tissues; the glands, hair follicles, and Malpighian layer will then stand out clearly.

4. Wash the section well in methylated spirit to remove all traces of the acid, then transfer to some perfectly clean and

strong methylated spirit for from ten to fifteen minutes to dehydrate.

5. Place some oil of cloves in a watch-glass, take the section from the spirit on a lifter, and carefully float it on to the surface of the oil, in which it must remain for about five minutes. This process is called clearing; the object of it is to remove the alcohol and to prepare the section for the balsam.

6. Transfer the section to some filtered turpentine to wash away the oil of cloves, and mount it in Canada balsam. Sections may be mounted in Canada balsam direct from the oil of cloves, but it is better to wash in turpentine first, because if much oil is mixed with the balsam it will not dry; the oil also has a tendency to cause the balsam to turn a dark yellow colour.

Ehrlich's Hæmatoxylin.—Hæmatoxylin, 30 grains; absolute alcohol, 3½ ounces; distilled water, 3½ ounces; glycerine, 3½ ounces; and ammonia alum, 30 grains. Dissolve the hæmatoxylin in the alcohol and the alum in the water; mix the two solutions together, and add the glycerine and 3 drachms of glacial acetic acid. The mixture must now be left exposed to light for at least a month, then filter and keep in a stoppered bottle.

Staining Process.—1. If the specimen has been hardened in any of the chromic solutions, place the section in a 1 per cent. watery solution of bicarbonate of soda for about five minutes, then wash well in distilled water. If it is a spirit preparation the soda will not be required, but all sections must be washed in distilled water before they go into hæmatoxylin stain.

2. To a watch-glassful of distilled water add from 10 to 20 drops of the hæmatoxylin solution, and immerse the section for from ten to thirty minutes.

3. Wash in distilled water, then in ordinary tap water; the latter will fix the dye and cause the colour to become blue.

When a section has been overstained with hæmatoxylin, the excess of colour may be removed by soaking it for a few minutes in a ½ per cent. solution of glacial acetic acid in distilled water, then wash again in tap water.

4. Dehydrate in methylated spirit.

5. Clear in clove oil, and mount in Canada balsam.

Double Staining with Hæmatoxylin and Eosin.—Stain the section in hæmatoxylin, as directed above, then place it in an alcoholic solution of eosin—about 1 grain of eosin to 1 ounce of methylated spirit is strong enough—and let it soak for about five minutes; wash well in methylated spirit, clear in clove oil, and mount in Canada balsam.

Ehrlich's hæmatoxylin is a good all-round stain, but, as it is acid, it must not be used for tissues of a mucous nature, such as the umbilical cord, and many tumours containing mucous or gelatinous tissues.

For these use **Delafield's hæmatoxylin** : To 400 c.c. of a saturated aqueous solution of ammonia alum add 4 grammes of hæmatoxylin dissolved in 25 c.c. of absolute alcohol; expose to the light and air in an unstoppered bottle for three or four days; filter, and add 100 c.c. of glycerine and 100 c.c. of wood spirit (methylic alcohol); allow the solution to stand in the light until it becomes of a dark colour, refilter, and keep in a stoppered bottle.

Use as directed for Ehrlich's stain.

Aniline Blue-Black.—Dissolve 30 grains of nigrosine in 3½ ounces of distilled water, then add 1 ounce of rectified alcohol and filter. This stain is only used for sections of brain and spinal cord. Immerse the sections for from thirty to sixty minutes, wash in water, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Aniline Blue.—Make a 1 per cent. solution of soluble aniline blue in distilled water and filter. Stain the section for five to ten minutes, wash in water, and place in methylated spirit, in which it must soak until the excess of colour is removed. Clear in clove oil and mount in Canada balsam.

This stain is useful for cardiac glands of the stomach, brain, and spinal cord.

Golgi's Nitrate of Silver Methods.—These are chiefly employed for investigating the relations of cells and fibres in the central nervous system. Two methods are mostly used, as follows :

(*a*) Very small pieces of the tissue, which have been hardened for some weeks in bichromate solution or Müller's fluid, are placed for half an hour in the dark in 0·75 per cent. nitrate of silver solution, and are then transferred for twenty-four hours or

more to a fresh quantity of the same solution (to which a drop or two of formic acid may be added). They may then be hardened with 50 per cent. alcohol, and sections, which need not be thin, are cut either from celloidin with a microtome or with the free hand. The sections are mounted in Canada balsam, which is allowed to dry on the slide. They must not be covered with a cover-glass, but the balsam must remain exposed to the air.

(b) Instead of being slowly hardened in bichromate, the tissue is placed at once in very small pieces in a mixture of bichromate and osmic (3 parts of Müller's fluid to 1 of osmic acid). In this it remains from two to five days, after which the pieces are treated with silver nitrate, as in the other case. This method is not only more rapid than the other, but is more sure in its results.

Mounting in Canada Balsam.—Take 3 ounces of dried Canada balsam and dissolve in 3 fluid ounces of pure benzol, filter, and keep in an outside stoppered bottle. Clear the section in clove oil, and place in turpentine. Clean a cover-glass and a slide, place a few drops of balsam on the centre of the latter, take the section from the turpentine on a lifter, allow the excess of turpentine to drain away, and with a needle-point pull the section off the lifter into the balsam on the slide. Now take up the cover-glass with a pair of forceps, and bring its edge in contact with the balsam on the slide ; ease it down carefully, so that no air-bubbles are enclosed, and with the points of the forceps press on the surface of the cover until the section lies quite flat, and the excess of balsam is squeezed out. The slide must now be put aside for a day or two to allow the balsam to harden ; the exuded medium may then be washed away with some benzol and a soft camel's-hair brush, after which dry the slide carefully with a cloth and apply a ring of cement. The above method answers well for mounting sections quickly, but when time will admit the following is a much better way : Clear the section and place it in turpentine ; clean a cover-glass, and moisten the surface of a slide with your breath ; apply the cover-glass to the slide, and make sure that it adheres. Place a few drops of balsam on the cover, into which put the section. Now put the slide away in a box, or in some place out of reach of dust, for twelve hours, so that the benzol may evaporate from the balsam. Clean a slide, warm it gently over the flame of a spirit-

lamp; apply a drop of balsam to the surface of the hardened
balsam on the cover-glass; take the cover up in a pair of forceps,
and bring the drop of fresh balsam in contact with the centre of
the warmed slide.   Ease the cover down carefully, so that no
air-bubbles may be enclosed, press on the surface of the cover-
glass until the section lies quite flat; set the slide aside to cool.
The exuded balsam may then be washed away with methylated
spirit and a soft rag, and a ring of cement applied.

Staining in Bulk.—Place small pieces of the tissue in
Grenacher's alcoholic carmine for from one to three days, then
transfer to a ½ per cent. solution of hydrochloric acid in methy-
lated spirit for from one to twelve hours, according to the size
of the tissue.   Wash well in spirit, and soak for a day in 90 per
cent. spirit.

The specimen may then be infiltrated and embedded in paraffin,
celloidin, or gelatine, but be careful to follow the instructions
previously given with each method.

Flemming's Method for Staining Karyokinetic Nuclei.—
Fix the tissue in the following Flemming's solution :

Osmic acid, 1 per cent. solution ...   ... 80 c.c.
Chromic acid, 10 per cent. solution   ... 15 „
Glacial acetic acid ...   . 10 „
Distilled water   ...   . 95 „

The fixing process is usually complete in twelve hours ; then
wash the tissue thoroughly in water and harden in alcohol of
gradually increasing strength.   Now place small shreds or thin
sections in a saturated alcoholic solution of saffranin mixed with
an equal quantity of aniline water for two days.   The tissue is
then to be washed in distilled water.   It is then soaked in abso-
lute alcohol until the colour is removed from everything except
the nuclei.   It is then again rinsed in water and placed in a
saturated watery solution of gentian violet for two hours, washed
again in distilled water, decolorized in alcohol until only the
nuclei are left stained ; then transfer to bergamot oil and mount
in xylol balsam.

Weigert-Pal Method for the central nervous system, by
which all medullated fibres are stained darkly, while the grey
substance and any sclerosed tracts of white matter are left

uncoloured.   Pieces of brain or spinal cord which have been hardened in Müller's fluid are to be placed direct in gum-water and syrup and soaked for a few hours ; then make sections with a freezing microtome, and place them in water, and from this transfer to Marchi's fluid, as follows :

Müller's fluid ... ... 2 parts,
Osmic acid, 1 per cent. ... ... 1 part,

and soak for a few hours.   They are then washed in water and transferred to the following stain : Dissolve 1 gramme of hæmatoxylin in a little alcohol, and add to it 100 c.c. of a 2 per cent. solution of glacial acetic acid, in which leave the section for twelve hours ; it will then be quite black.   Wash again in water, and place in a ¼ per cent. solution of potassic permanganate for five minutes ; rinse with water and transfer to Pal's solution (sulphate of soda, 1 gramme ; oxalic acid, 1 gramme ; distilled water, 200 c.c.), and bleach for a few minutes.   When sufficiently bleached they are passed through water into alcohol, cleared in bergamot oil, and mounted in Canada balsam.

Ehrlich's Triple Stain for Blood-Corpuscles.

Saturated watery solution, orange ' G ' ... 135 parts.
        ,,   ,,   ,,   methyl green ... 110   ,,
        ,,   ,,   ,,   acid fuchsin ... 100   ,,

To the above add—

Glycerine ... . 100 parts.
Absolute alcohol 200   ,,
Distilled water 300   ,,

This solution should stand for several weeks to allow for sedimentation, and it improves with age.   When used the supernatant liquid should be drawn off with a pipette to avoid the sediment.

The cover-glasses are to be well cleaned with alcohol, and the surface of one is touched with a drop of fresh blood, and another cover-glass pressed on its surface until the blood is evenly distributed.   The covers are then separated and allowed to dry.

When dry they must be still further hardened over a spirit-lamp, or on a hot stage made of sheet copper, and kept at 212° F. for from fifteen minutes to two hours; after which place in stain for from one to four minutes, wash in water, dry, and mount in Canada balsam, benzol, or xylol.

The eosinophile granules in the corpuscles will be a reddish hue, the neutrophile granules purple, and the nuclei bluish-green or blue.

Toison's Solution for Staining White Blood-Corpuscles.

Methyl violet ...	...	...	...	$\frac{1}{2}$ grain.
Neutral glycerine	...	...	...	1 ounce.
Distilled water ...	...	...	...	$2\frac{1}{4}$ ounces.

Mix thoroughly and add—

Chloride of sodium	...	...	...	15 grains.
Sulphate of sodium	...	...	...	2 drachms.
Distilled water ...	...	...	...	$5\frac{1}{2}$ ounces.

Filter and keep in a stoppered bottle.   Spread blood on cover-glass, dry, and immerse in stain for eleven minutes.   Wash in water, dry and mount in Canada balsam.

Fixing and Staining Sections on the Slide.

Mayer's Albumen Method.—White of egg, 50 c.c.; glycerine, 50 c.c.; salicylate of soda, 1 gramme: shake well togther, and filter into a stoppered bottle.   A thin layer of the cement is spread on a slide with a brush, and the section laid on it.   Now warm gently on a water-bath.   As the paraffin melts it is carried away from the section by the albumen.   The section may now be washed with turpentine, benzole, and alcohol, and be treated with aqueous or other stains, without fear of it moving.

Shellac Method.—Make a solution of shellac in absolute alcohol —it should be about the thickness of oil—filter, and keep in a stoppered bottle.   Warm some slides, and spread over them a layer of the cement with a brush, and put away to dry.   When dry apply a very thin layer of creosote; this will form a sticky

surface, on which the section must be carefully laid. Now heat the slide on a water-bath for about fifteen minutes at the melting-point of the paraffin ; this will allow the section to come down on the shellac film, and at the same time evaporate the creosote. Allow the slide to cool, and wash away the paraffin with turpentine or benzol. If the section has been stained in bulk, a drop or two of Canada balsam is added, and a cover-glass applied.

To Stain a Section on the Slide.—Fix section on slide as directed above. Wash away the paraffin with rectified mineral naphtha, follow this quickly with a few drops of methylated spirit, and then with some distilled water. Now apply the stain, and place the slide under a bell-glass to prevent evaporation ; or the slide may be plunged into a vessel containing the staining solution. When sufficiently stained, wash with distilled water, dehydrate with methylated spirit, drain away the spirit, and apply a drop of clove oil to clear the specimen. When clear, drain away as much of the oil as possible, add a drop of Canada balsam, and apply the cover-glass.

Lesson IV.

STAINING BLOOD AND EPITHELIUM, TEASING - OUT TISSUES, AND MOUNTING IN AQUEOUS MEDIA STAINING WITH PICROCARMINE, GOLD CHLORIDE, SILVER NITRATE, AND OSMIC ACID.

Double Staining Nucleated Blood-Corpuscles.

Stain A.—Dissolve 5 grains of eosin in $\frac{1}{2}$ ounce of distilled water and add $\frac{1}{2}$ ounce of rectified alcohol.

Stain B.—Dissolve 5 grains of methyl green in an ounce of distilled water.

Place a drop of frog's blood on a slide, and with the edge of another slide spread it evenly over the centre of the slip; now put it away out of reach of dust to dry. When quite dry, flood the slide with Stain A for three minutes. Then wash with water, and flood the slide with Stain B for five minutes. Wash again with water, and allow the slide to dry. Apply a drop or two of Canada balsam and a cover-glass.

Blood of Mammals, Non-Nucleated Corpuscles.

Spread a drop of blood on a slide and let it dry for twelve hours, then stain in a strong alcoholic solution of eosin for about five minutes, drain away the eosin, rinse the slide in methylated spirit, let it dry, apply a drop of Canada balsam and the cover-glass.

Both of the above processes should be carried out during dry weather, as any moisture in the air retards the drying of the corpuscles, and then they are liable to change their form.

Epithelium.—Kill a frog, cut off its head, and remove the lower jaw. Open the abdomen and take out the stomach, and slit it open. Place the head, jower jaw, and stomach in a 2 per cent. solution of bichromate of potash for forty-eight hours. Then wash gently in water until no colour comes away from the specimens. Now place all three portions in picrocarmine for twenty-four hours. Remove the tissues from the carmine, and allow the stain to drain away from them. Take the lower jaw and scrape the tongue for squamous epithelium, and place the deposit obtained in a few drops of glycerine on a slide. Take the stomach, remove some columnar epithelium from its internal surface, and place it in some glycerine on another slide. Then take the head for ciliated epithelium, which will be found at the hinder part of the roof of the mouth ; put some scrapings from this in glycerine on a slide as before. Clean a slide and place a drop or two of Farrant's medium on its centre ; take up a little of the epithelium on the point of a needle, and put it into the medium. Now apply a cover-glass, and with the needle-point press it down until the epithelial cells are separated and spread evenly between the cover and the slide. Put the slide aside for a day or two, so that the medium may set. Then wash away the excess of medium with some water and a camel's-hair brush, dry the slide with a soft rag, put it on a turn-table, and run on a ring of cement.

Portions of the tongue, trachea, and intestine of a rabbit or cat may be treated in the same way.

Endothelium.—Take a piece of the omentum of any small animal, and rinse gently in distilled water to remove soluble matter. Place it in a $\frac{1}{4}$ per cent. solution of silver nitrate for ten

minutes, or until it becomes a milky white. Wash well in ordinary water, and expose in a saucer of water to diffused sunlight, until it assumes a brownish colour. Cut out a small piece and mount it in Farrant's medium or glycerine jelly. In this specimen only the interstitial cement substance will be seen. To compare with it, cut out a similar piece, wash it in distilled water, and stain it with hæmatoxylin for ten minutes ; wash away all excess of stain with distilled water, and mount in Farrant's medium or glycerine jelly. In this specimen the nuclei will be seen stained blue. Specimens of mesentery showing endothelium may also be mounted in Canada balsam. When this is desired, stain the tissue as directed above, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Teasing-out Tissues.—Take a very small piece of the tissue, place it on a slide in a few drops of distilled water, and with a couple of needles mounted in holders carefully separate the fibres from each other. When the parts are sufficiently isolated, drain away the water, add a few drops of the mounting fluid, and apply the cover-glass. When teasing it is very important that a proper background should be used so that the object may be easily seen. For a coloured specimen, a piece of white paper should be used, and a transparent white tissue will be seen better on a dark ground, such as a piece of black paper or American cloth ; the slide should be examined from time to time under the low power of the microscope to ascertain when the tissue is teased out enough.

White Fibrous Tissue.—Harden some tendons from a rat's tail in methylated spirit for a week. Then soak a small piece in water to remove all trace of spirit, place it on a slide in a few drops of water, and tease it out until the fibres are separated from each other. Drain away the water, add some Farrant's medium or glycerine jelly, and apply a cover-glass.

Yellow Elastic Tissue.—Place small pieces of the ligamentum nuchæ of an ox in chromic acid and spirit for ten days. Then proceed as above.

Striped or Voluntary Muscle.—Harden small pieces of muscle of a pig in a 2 per cent. solution of bichromate of potash for three weeks, then transfer to methylated spirit, in which it may remain until required. Soak a piece in water to remove the

spirit, place a very small fragment on a slide in a few drops of water, and with a couple of needles tease or tear the tissue up so as to separate the fibres. Drain away the excess of water, apply a drop or two of Farrant's medium or glycerine jelly and a cover-glass.

Non-Striped Muscle.—Harden a piece of the intestine of a rabbit in chromic acid and spirit for ten days. Wash in water, strip off a thin layer of the muscular coats, and stain it in hæmatoxylin. Wash in distilled water, and then soak in ordinary tap-water until the colour becomes blue. Clean a slide, put a small fragment of the muscle on it in a few drops of water, and with needles separate the fibres. Drain off the excess of water, apply a few drops of Farrant's medium or glycerine jelly and a cover-glass.

Nerve Fibres.—Dissect out the sciatic nerve of a frog, and stretch it on a small piece of wood as follows: Take a match, make a slit in each end of it, into which put the ends of the nerve; now place it in a 1 per cent. solution of osmic acid for an hour or two. Wash in water, tease out a small fragment on a slide, and apply a few drops of Farrant's medium or glycerine jelly and a cover-glass.

When staining with gold chloride, solutions from $\frac{1}{2}$ per cent. to 5 per cent. in distilled water are employed. It is used for staining nerves and nerve-endings; it also brings out the cells of the cornea, fibrous connective tissues, and cartilage.

The tissue must be taken from the animal immediately after death, and be placed in the solution of gold for from half an hour to an hour; it is then removed to distilled water for twelve hours, and afterwards exposed to the action of diffuse sunlight in a saturated solution of tartaric acid or formic acid until it assumes a purple colour.

The future treatment will depend on the nature of the specimen.

If muscle has been stained for nerve-endings, place a small piece on a slide, tease it up, and examine with a low power until you find a nerve-fibre terminating in an end-plate on a muscle-fibre, separate it from the surrounding fibres as much as possible, add some Farrant's medium or glycerine jelly, and apply a cover-glass.

If cornea or cartilage, make vertical and horizontal sections

with a freezing microtome, and mount in Farrant's medium or glycerine jelly. Sections of gold-stained tissues may also be mounted in Canada balsam; when this is desired, dehydrate in strong spirit, clear in clove oil and mount in Canada balsam.

There are many ways of staining with gold, but the above is the most simple, and it gives very good results. For the other methods the student may refer to the larger works on practical histology.

Staining with Picrocarmine.—Rub up 1 gramme of carmine with 10 c.c. of water, and 3 c.c. of strong liquid ammonia; add this to 200 c.c. of a saturated solution of picric acid in distilled water. Leave the mixture exposed to the air until it evaporates to one-third of its bulk; filter, and keep in a stoppered bottle. Place some of the picrocarmine in a watch-glass, and immerse the section for from half an hour to an hour. Remove from the stain with a lifter, and place the section on a slide; drain away as much of the excess of stain as possible, and, if necessary, soak up what remains with a piece of filter-paper. Then add a few drops of Farrant's medium, and apply the cover-glass.

Picrocarmine stained tissues should never be washed; if they are, all the yellow colour will be removed, and the specimen will come out stained with carmine only. They improve by keeping, and the staining process goes on for several days after they are mounted; that is to say, some parts give up the stain, and others absorb it. Picrocarmine may be purchased in crystals, with which a 2 per cent. solution in distilled water should be made.

If it is desired to mount a picrocarmine stained section in Canada balsam proceed to stain as above; then make a saturated solution of picric acid in methylated spirit, filter, and dehydrate the section in it; then give it a final rinse in methylated spirit, clear in clove oil and mount in Canada balsam.

Farrant's Medium. — Take of glycerine and a saturated aqueous solution of arsenious acid equal parts, and mix them well together; then add as much powdered gum arabic as the mixture will take up, and let it stand for six weeks. Filter, and keep in an outside stoppered bottle.

The above is difficult to make; it is better to obtain it ready for use.

Glycerine Jelly.—Dissolve 1 ounce of French gelatine in

6 ounces of distilled water; then melt in a water-bath, and add 4 ounces of glycerine and a few drops of creosote or carbolic acid. Filter through paper while warm, and keep in a stoppered bottle. The above may be used instead of Farrant's medium. The jelly must, of course, be warmed before use. All tissues or sections must be well soaked in water before they are mounted in Farrant's medium or glycerine jelly, so that all trace of alcohol is removed.

Tissues containing much air should be soaked in water that has been boiled for about ten minutes and allowed to cool.

<center>LESSON V.</center>

INJECTION OF BLOODVESSELS—PREPARATION OF HARD TISSUES.

Injection of Bloodvessels.

Carmine and Gelatine Injection Mass.—

Pure carmine	60 grains.
Liq. ammonia fort.	2 drachms.
Glacial acetic acid	86 minims.
Gelatine solution (1 ounce in 6 ounces	
of water)	2 ounces.
Water	2 ounces.

Dissolve the carmine in the ammonia and water in a test-tube, and mix it with one half of the warm gelatine. Add the acid to the remaining half of gelatine, and drop it little by little into the carmine mixture, stirring well all the time with a stick or glass rod. Filter through flannel, and add a few drops of carbolic acid to make the mass keep. The principle to be remembered in making the mass is this: the carmine, if alkaline, would diffuse through the vessels and stain the tissues around them; if acid, the carmine would be deposited in fine granules, which would block up the capillaries; hence the necessity for a *neutral* fluid. The best guides are the colour and smell of the fluid. It should be a bright red, and all trace of the smell of ammonia neutralized. The gelatine solution is made by putting

1 ounce of gelatine into 6 ounces of water; it must then be left until the gelatine becomes quite soft; then dissolve over a water-bath.

Prussian or Berlin Blue and Gelatine Mass.—Take 1½ ounces of gelatine, place it in a vessel and cover it with water; allow it to stand until all the water is absorbed and the gelatine is quite soft. Then dissolve in a hot-water bath. Dissolve 1 drachm of Prussian or Berlin blue and 1 drachm of oxalic acid in 6 ounces of water, and gradually mix it with the gelatine solution, stirring well all the time; then filter through flannel.

Watery Solution of Berlin Blue.—Dissolve 2½ drachms of the blue in 18 ounces of distilled water, and filter. The fluid is useful for injecting lymphatics.

Injecting Apparatus Required.—An injecting syringe fitted with a stop-cock, and several cannulæ of various sizes.

Directions for Injecting.—The animal to be injected should be killed by chloroform, so that the vessels may be dilated, and injected while warm ; if possible it should be placed in a bath of water at a temperature of 40° C. Expose the artery of the parts to be injected, clear a small portion of it from the surrounding tissues, and place a ligature of thin twine or silk round it. With sharp scissors make an oblique slit in the wall of the vessel, insert the cannula, and tie the ligature firmly over the artery behind the point of the cannula, into which put the stop-cock. Fill the syringe with injection-fluid, which must not be too warm, and take care not to draw up any air-bubbles; now insert the nozzle of the syringe into the stop-cock and force in a little fluid ; remove the syringe, so that the air may escape, insert the syringe again, and repeat the process until no air-bubbles come out of the stop-cock. You may then proceed slowly with the injection. Half an hour is not too long to take over the injection of an animal of the size of a cat. The completeness of an injection may be judged by looking at the vascular parts, such as the tongue, eyelids, and lips. When the injection is complete shut the stop-cock, remove the syringe and cannula, and tie the ligature round the artery. Now place the animal in cold water for an hour to set the injection-fluid. When quite cold, dissect out the organs, cut them up into small pieces, and place them in methylated spirit to harden, and change the spirit every twenty-

four hours for the first three days.   The hardening will be complete in ten days.

Injection of Lymphatics (*Puncture Method*).—A small sub-cutaneous syringe is filled with a watery solution of Berlin or Prussian blue, and the nozzle is thrust into the pad of a cat's foot.   The injection is to be forced into the tissues.   Then rub the limb from below upwards.   This will cause the injection-fluid to flow along the lymphatics and find its way into the glands of the groin.

To Inject Lymph-Sinuses of Glands.—Force the nozzle of a subcutaneous syringe into the hilum of a lymphatic gland of an ox, and inject a watery solution of Prussian or Berlin blue until the blue appears on the surface of the gland.   Then place it in methylated spirit to harden.

When *blue* injection-fluid is used, add a few drops of acetic acid to the spirit while hardening the tissues.

CUTTING, GRINDING, AND MOUNTING SECTIONS OF HARD TISSUES.

Bone.—Take the femur of a sheep, remove as much of the muscle as possible, and macerate in water until quite clean, then allow it to dry.

1. With a fine saw make transverse and longitudinal sections.

2. Take a hone (water of Ayr stone), moisten it with water, and rub one side of the section upon it until it is quite flat and smooth.

3. Wash in water, and set aside until quite dry.

4. Take some dried Canada balsam, place a piece on a square glass, and warm gently over a lamp until the balsam melts; allow it to cool a little, and then press the smooth side of the section into it, and set aside until cold.

5. With a fine file rub the section down as thinly as possible.

6. Take the hone again and grind the section down until thin enough, using plenty of water.

7. Place it, with the glass, in methylated spirit until the section comes away from the glass, then wash well in clean water and allow to dry.

8. Place the section on a slide, and apply a very thin coat of gum water to its upper surface, taking great care that the gum

does not run under the section, and let it dry. This coat of gum will hide any fine scratches that may be left on the section. Now take a thin cell just deep enough for the section, and apply a coat of cement to its upper edge; place the section in its centre with the gummed side uppermost, and apply the cover-glass, which should come down on the bone to keep it in the centre, hence the necessity of a cell of just the proper depth. The object of a section of dry bone is to show the canaliculi; when mounted in fluid of any kind these are obliterated. Sections of teeth are made in the same way.

Lesson VI.

CUTTING, STAINING, AND MOUNTING VEGETABLE SECTIONS.

Stems, leaves, roots, etc., should be hardened in methylated spirit for a week or ten days, and the spirit changed every twenty-four hours for the first three days. The stems must not be too old. One, two, and three years' growth will show all that is required.

Wheat, barley, maize, peas, etc., are usually obtained dry. They must be placed in water for a few hours or until they resume their natural shape. Then lay a piece of blotting-paper on a plate, moisten it with water, and spread a layer of the grains on its surface; now place another piece of wet blotting-paper over all, and put in a warm place for from twelve to twenty-four hours, so that the embryo may begin to germinate. Then remove from the plate, and place the grains in a bottle of methylated spirit, which must be changed every day until all trace of water is removed. The specimens may then be section-ized, or they may remain in spirit until required.

Ovaries.—Gather some before the flower opens, and others after it has been open for a day. You will then have the ovules in both stages. Place them in methylated spirit and change every twenty-four hours for the first three days.

Anthers.—Treat in exactly the same way as ovaries, but anthers must be infiltrated with celloidin before the sections can

be cut. Remove the ends, place in equal parts of alcohol and ether, and soak for twelve hours ; then place in celloidin, and, after soaking for from twelve to twenty-four hours, proceed as directed in Lesson II. on Section-Cutting.

Some specimens after being in spirit are too hard to cut easily. They may be softened by soaking in warm water. Leaves are often particularly troublesome in this respect; they bend and become fixed by the action of the spirit, and will not then stand the slight pressure required to hold them firmly between the carrot without cracking. When this happens, soak the leaf in warm water until it is quite pliable; it can then be embedded in carrot without any risk of being broken. Stems and petioles of many palms are naturally too hard, and they may contain a large amount of silica. They must be soaked in water for a while; then transfer to liq. potassæ for from one to twelve hours. Wash again well in water to remove all trace of potash, then reharden in methylated spirit. The shells of many stone fruits may be softened and cut by this method.

Section-Cutting, by hand and with a microtome, should be done in the same manner as described in Lesson II.

Bleaching.—Vegetable sections generally require bleaching before they can be properly stained. Chlorinated soda is used for this purpose. Take of dry chloride of lime, 2 ounces ; of washing soda, 4 ounces ; and distilled water, 2 pints. Mix the lime in one pint of the water and dissolve the soda in the other. Mix the two solutions together, shake well, and let the mixture stand for twenty-four hours. Pour off the clear fluid, filter, and keep in a stoppered bottle in a dark place, or cover the bottle with paper. Soak the sections in distilled water. Pour off the water and add a quantity of bleaching fluid. Allow this to act for from one to twelve hours. Wash well in water, which must be changed several times to remove all traces of soda. The sections may now be stained, or they may be preserved in spirit until required.

Staining with Borax Carmine (suitable for ovaries, fruits, etc.).—Pure carmine, 1 drachm ; liq. ammoniæ fort., 2 drachms. Dissolve the carmine in the ammonia, and 12 ounces of a saturated solution of borax in distilled water. Filter and keep in a stoppered bottle.

1. Put some stain in a watch-glass, and immerse the section for three to five minutes.

2. Wash well in methylated spirit.

3. Take of hydrochloric acid, 1 part; and of methylated spirit, 5 parts. Mix well together, and soak the section until the colour changes to a bright scarlet, which takes about five minutes. The acidulated spirit may be kept ready for use at any time.

4. Wash well in methylated spirit. Then place in some strong methylated spirit, and soak for at least ten minutes to dehydrate.

5. Place the section on the surface of a small saucer of clove oil, and let it soak until clear.

6. Remove from the clove oil and place in turpentine, and then mount in Canada balsam.

Full instructions for mounting in Canada balsam are given at end of this lesson.

Hæmatoxylin.—Hæmatoxylin, 30 grains; absolute alcohol, $3\frac{1}{2}$ ounces; distilled water, $3\frac{1}{2}$ ounces; glycerine, 3 ounces; ammonia alum, 30 grains; glacial acetic acid, 3 drachms. Dissolve the hæmatoxylin in the alcohol and the alum in the water; then add the glycerine and acetic acid. Mix the two solutions together and let the mixture stand for at least a month before use.

1. Add about thirty drops of the above to an ounce of distilled water, and stain the section for fifteen to thirty minutes.

2. Wash well in distilled water, and then in ordinary tap water. This will fix the colour and make it deeper.

3. Dehydrate in strong methylated spirit for at least ten minutes.

4. Clear in clove oil and mount in Canada balsam.

Double Staining—Dalton Smith's Method.—Stems, roots, and leaves:

Green Stain.—Acid aniline green	...	2 grains.
Distilled water	...	3 ounces.
Glycerine ...	...	1 ounce.

Mix the water and glycerine together, and dissolve the green in the mixture.

Carmine Stain A.—Borax   ...   ...   10 grains.
                  Distilled water   ...   1 ounce.
                  Glycerine   ...   ...   ½   ,,
                  Alcohol rect.   ...   ½   ,,

Dissolve the borax in the water, and add the glycerine and alcohol.

Carmine Stain B.—Carmine   ,..   ...   10 grains.
                  Liq. ammoniæ   ...   20 minims.
                  Distilled water   ...   30 minims.

Dissolve the carmine in the water and ammonia.   Mix A and B together, and filter.

1. Place the section in green stain for five to ten minutes. ·
2. Wash in water.
3. Place in carmine from ten to fifteen minutes.
4. Wash well in methylated spirit.
5. Dehydrate and clear in clove oil.   Wash in turpentine and mount in Canada balsam.

Double Staining—M. J. Cole's Method.—

Pure carmine ...   .   1 drachm.
Liq. ammonia ...   .   2 drachms.

Dissolve the carmine in the ammonia and add 12 ounces of a saturated solution of borax in distilled water.   Filter through paper and keep in a stoppered bottle.

Bleach the sections, and after being well washed with repeated changes of water they are placed in the above stain for five to ten minutes.   Then wash well in methylated spirit, and soak in acidulated alcohol—1 part hydrochloric acid to 35 of methylated spirit—until the excess of stain is removed ; about two minutes is usually sufficient.   Wash again in methylated spirit to remove all trace of acid.   Dissolve 5 grains of acid aniline green in 6 ounces of methylated spirit, and filter if necessary.   Soak the section in this green stain for at least half an hour; then just rinse in methylated spirit, clear in clove oil, and mount in Canada balsam.   The advantage of this method is that the section can remain in the green stain for any time.   The

writer keeps a stock of sections in it ready for mounting. Should a specimen be overstained green, the excess of colour can easily be removed by soaking in methylated spirit for a few minutes.

Staining with Eosin.—Make a 2 per cent. solution of eosin in alcohol, filter if necessary, and keep in a stoppered bottle. This stain is used for showing the structure of sieve-tubes and plates; it stains protoplasm deeply. Make transverse and longitudinal sections of the stem of a vegetable marrow, and immerse them in the above for ten minutes. Then wash out any excess of colour with methylated spirit, clear in clove oil, and mount in Canada balsam.

Staining Hairs on Leaves.—Make a 2 per cent. aqueous solution of soluble aniline blue, and filter. Now take, for example, a young leaf of *Deutzia scabra*, cut it into small pieces of about ¼ inch square, and bleach in chlorinated soda. Then wash well in water, and immerse in the above stain for twelve hours, wash well in water, and transfer to methylated spirit, in which they must be soaked until nearly all the colour is removed. Then soak in clove oil for several hours, and when quite clear mount in Canada balsam.

Leaves of eucalyptus and other plants showing essential oil glands may be treated in the same way, but if the specimens have been preserved in spirit they must be soaked in water before the bleaching process.

Male and Female Conceptacles of Fucus and Other Algæ.—Place the specimens in methylated spirit, which must be changed every twenty-four hours for the first three days, then let them soak for ten days or until required for cutting into sections.

Embed a conceptacle in carrot, place in microtome, and make transverse sections, which must be as thin as possible. While cutting, keep the knife well wetted with methylated spirit, and, as the sections are cut, put them into spirit; no water must come near them. When ready, stain the sections in a strong solution of acid aniline green in spirit for several hours. Then just rinse in absolute alcohol, clear in clove oil, and mount in Canada balsam.

Transverse and longitudinal sections of the thallus of an alga

may be treated in the same way, but they may be mounted without staining, as the tissues are coloured naturally brown and yellow.

Ovaries of Flowers.—Make transverse sections, which should be as thin as possible, and stain them either in borax carmine or hæmatoxylin, then clear in clove oil, and mount in Canada balsam.

Anthers must be infiltrated with celloidin to keep the pollen in position.   Then embed in carrot, place in microtome, and cut transverse sections.   Stain in borax carmine, and after having passed through acidulated spirit, wash well in methylated spirit, and dehydrate for about one to two minutes in absolute alcohol; then clear in oil of origanum or bergamot, and mount in Canada balsam.

Flower Buds. — Infiltrate with celloidin as directed in Lesson II. on Section-Cutting.   Embed the specimen in carrot, and place in the microtome.   Cut transverse sections, stain in borax carmine, and pass through acidulated spirit to remove excess of colour ; if desired, they may be soaked until the stain is removed from everything except the nuclei.   Wash well in methylated spirit, and place in absolute alcohol for from one to two minutes ; then clear in oil of origanum or bergamot, and mount in Canada balsam.   Great care must be taken that the sections do not remain too long in absolute alcohol; if they should, the celloidin will dissolve, and the sections will fall to pieces.

Pollens.—Place some mature anthers in a large pill-box, and allow them to become perfectly dry.   Shake the box well until all the pollen is set free; then remove the anther sacs with a pair of forceps, and place the pollen in a bottle of turpentine; soak for several days to remove all trace of air, then pour off the turpentine; take up a little of the pollen on the point of a penknife, and place it in a few drops of Canada balsam on a cover-glass ; stir up with a needle to spread the grains evenly over the cover, and put away to dry.   When the balsam has dried, add a few more drops of balsam, take up the cover with a pair of forceps, and mount it on a warm slide.   This method of mounting must always be employed for pollens, because, if they are put up in any other way, the balsam only hardens at the edge of the cover, and remains in a more or less fluid state

in the centre, with the result that, if the slide were placed on its edge, the specimens would run together in a heap at the lower side of the cover.

Pollens may also be mounted as opaque objects (see Dry Mounts, p. 307).

Pollens may also be stained various colours by aniline dyes. Place some fresh pollen in methylated spirit, and soak until air and most of the colour is removed. Then pour off the spirit and add a strong alcoholic solution of some aniline dye of the desired colour ; any will do so long as it is soluble in alcohol. Soak in the dye for an hour or two, then pour off the stain, just rinse in spirit, pour this away, and add clove oil, and when clear pour off the oil ; take up a little pollen on the point of a knife, and mount in Canada balsam as directed for unstained specimens.

Specimens of pollens are sometimes stained many colours on the same slide. This is done in the following way : Take some pollen and divide it into equal quantities, each one of which is to be stained in a different dye. Then when they have been cleared by the clove oil they are all mixed together and mounted in balsam.

Pharmacological Specimens.—Students of pharmacy may desire to make sections of the dried stems, roots, and leaves with which they deal. Place the dry specimen in water, and soak until it resumes as nearly as possible its natural shape. Then place in methylated spirit, which must be changed every twenty-four hours for three days to remove all the water. Then make sections in accordance with instructions given for ordinary botanical specimens.

Powdered Drugs.—Place some of the powder in methylated spirit, and soak for an hour or two ; then pour off the spirit, and add clove oil ; let it stand a little while, then drain off the oil, take up some of the powder on the point of a knife, place it in some Canada balsam on a cover-glass, mix it well up with the balsam, and then proceed to mount it as directed for pollens.

Some specimens may not be suitable for mounting in Canada balsam ; they should then be mounted in glycerine jelly. Mix the powder with water, and soak until all trace of air is removed; allow the mixture to settle down, then pour off the water, take up a small quantity of the deposit, and mount in glycerine

jelly as directed for starches in Lesson VII. Sometimes neither glycerine jelly nor balsam will suit ; then mount dry.

Make an opaque cell, place a little patch of gum-water on its centre ; allow this to dry, then moisten the patch of gum with your breath. Fill the cell with the powder, and let it stand for a minute or two ; then shake out the powder that has not adhered, and apply a cover-glass.

Mounting in Canada Balsam.—Take 3 ounces of dried Canada balsam and dissolve in 3 fluid ounces of benzole. Filter and keep in an outside stoppered bottle.

1. Clean a cover-glass, moisten the surface of a slide with the breath, apply the cover-glass to it, and make sure that it adheres.

2. Place a few drops of balsam on the cover-glass.

3. Take the section out of the turpentine on a lifter, and put it into the balsam on the cover.

4. Put away out of the reach of dust for twelve hours to allow the benzole to evaporate from the balsam.

5. Warm a slide over a spirit-lamp and apply a drop of balsam to that on the cover-glass ; take it up with a pair of forceps, and bring the drop of fluid balsam in contact with the centre of the warmed slide. Ease the cover down carefully, so that no air-bubbles may be enclosed, and press it down with the point of the forceps until the section lies quite flat and the excess of balsam is squeezed out. Allow the slide to cool, and the excess of balsam may then be washed away with some methylated spirit and a soft rag.

Lesson VII.

THE PREPARATION OF VEGETABLE TISSUES FOR MOUNTING IN GLYCERINE JELLY, ACETATE OF COPPER SOLUTION, ETC.

Epidermis for Stomata.—Take a leaf, remove the edges with a pair of scissors, and then cut the remainder up into small pieces of about ¼ inch square. Place these in a test-tube, add nitric acid, and boil gently over a spirit-lamp for about a minute, then add a few grains of chlorate of potash, and bring to the boiling-point again. Pour away the acid and add water, which must be changed several times until all trace of acid is removed.

The epidermis will then be found quite clean, and it may be stained and mounted at once, or be placed in spirit and kept until required.

Another Way.—Some epidermal tissues are very delicate and will not stand the acid treatment. When this is the case cut the leaf up as directed above, place the pieces in a jar of water, and put aside for a week or two. The action of water will rot the cellular tissue and set the epidermis free. Then wash well in water, and should any particles of débris adhere, they can be removed by brushing with a camel's-hair brush.

The epidermis of some plants will not stand either of the above processes. When this is the case, the only plan is to strip off a small piece of the cuticle, lay it on a slide, inner side uppermost, and with a scalpel carefully scrape away cellular tissue that may be adhering. Then wash in water, and proceed with the staining.

To Stain the Epidermis.—Make a 1 per cent. solution of methyl aniline violet in distilled water and immerse the specimen for about five minutes. Then wash in a ½ per cent. solution of glacial acetic acid to remove excess of colour, wash away all trace of acid with water and mount in glycerine jelly in the following way :

Warm the jelly carefully in a water-bath until it is quite fluid. Warm a slide, take up a little jelly in a dipping-tube, and place it on the slide; now take up the epidermis with a lifter and put into the jelly on the slide, being very careful to avoid making any air-bubbles. Now take the cover-glass and apply it to the surface of the jelly, push down the cover with the point of a needle until it is quite flat, and then set aside to cool. The above process applies to all specimens that are to be mounted in jelly ; but when tissues have been preserved in spirit they must be soaked well in water before being mounted.

Annular Vessels.—Get some stem of maize, cut it into pieces about ½ inch long, and then cut again into thin longitudinal slices ; place these in water until rotten. Now put some of the broken-up material on a slide and examine with a microscope ; pick out the annular vessels on the point of a needle, place them in some clean water, and wash well. Stain in a weak watery solution of acid green, and after washing in water, mount in glycerine jelly.

Scalariform Vessels.—Treat pieces of the rhizome of *Pteris aquilina* in exactly the same way as stem of maize.

Spiral Vessels.—Treat pieces of the stem of rhubarb in the same manner as annular vessels.

Raphides may be isolated, or they can be mounted *in situ*, in the tissues in which they occur. For the former, take some leaves of cactus, stem of rhubarb, and root of Turkey rhubarb, cut them up into thin slices longitudinally, and place them in a jar of water, covered up to keep out dust, and put away until the tissue has become perfectly disintegrated. This will take several weeks, and the process is more easily carried out by keeping the jar in a warm place. When all the material has broken up, stir well with a glass rod, and strain through a piece of coarse muslin into a shallow vessel, such as a soup plate ; stir up again, and then allow to settle for a minute, so that the raphides may fall to the bottom of the plate ; now pour away as much of the dirty water as possible, add more clean water, and repeat the process until you have got rid of all the disintegrated vegetable fibre. Now pour the raphides into a bottle, and if they are quite clean, pour off the water and add methylated spirit, in which they may be preserved until required for mounting.

To mount isolated raphides, clean a cover-glass, fasten it to a slide with the aid of your breath, take up some of the raphides in a dipping-tube, place them on the cover-glass, and spread them evenly over its surface with a needle. Place the slide out of reach of dust until all the spirit has evaporated, and the raphides are quite dry ; add a few drops of Canada balsam, and put the slide away again for twelve hours ; then add a few drops more balsam, take up the cover with a pair of forceps, and mount it on a warmed slip. When the raphides are very large they must be mounted in balsam that is rather thicker than is usually used.

Raphides in situ in Tissues.—Harden the stems, roots, or leaves in methylated spirit, and make sections in the ordinary way; dehydrate, clear in clove oil, and mount in Canada balsam.

Raphides in Scale-Leaves of Bulbs, such as Onion, Garlic, Lily, Hyacinth.—Strip off a thin portion of the cuticle, place it in methylated spirit for a few hours, and when dehydrated clear in clove oil and mount in Canada balsam.

Sometimes raphides are rendered too transparent when mounted in balsam. When this is the case they must be put up in glycerine jelly in the following way :

Isolated Specimens.—Pour off the methylated spirit, and add water ; pour off the water, leaving the raphides at the bottom of the bottle. Clean a cover-glass and a slide. Place a few drops of warmed glycerine jelly on the centre of the slide ; take up a few of the raphides on the point of a penknife, and place them in the glycerine jelly, but do not stir them up. Now apply the cover-glass, and press it down carefully with a needle, giving it at the same time a twisting motion, to spread the raphides evenly between the cover and slide. Put away for an hour or two, scrape off the excess of jelly with a penknife, wash in water, and then in methylated spirit, dry with a cloth, and apply a coat of black enamel. When raphides in the tissues are prepared in glycerine jelly, wash away all trace of spirit with water, and mount in glycerine jelly as above.

Starches (*Isolated Specimens*).—If the tissue is fresh, scrape the cut surface with a knife, and place the scrapings in a bottle of water ; shake well and then strain through fine muslin into a shallow vessel ; let the starch settle, pour off the water, and wash again with some clean water until the starch is quite clean ; then place it in a bottle, and when it has settled to the bottom, pour off the water, and add methylated spirit.

Dried Specimens.—Place in water until the tissue swells up, then, if the material is large enough, it may be scraped and treated as above. If too small—small seeds, for instance—place them in a mortar in some water, and carefully break them up ; strain through muslin, wash with water until quite clean, and preserve in methylated spirit.

Starches may be mounted in Canada balsam or glycerine jelly. If the former is chosen, spread a little starch evenly on a cover-glass, let it dry, apply some Canada balsam, and mount it in the ordinary way. For glycerine jelly pour off the spirit and add water, then allow the starch to settle to the bottom of the bottle ; pour away the water. Place a few drops of glycerine jelly on a slide, take up some starch on a penknife, and place it in a little heap in the jelly ; now apply a cover-glass, and press down with a gentle twisting movement until the starch is evenly spread.

Let the jelly set, scrape away the excess, wash in water, then in spirit, dry, and apply a coat of cement.

It is desirable also to prepare specimens of starch *in situ* in the tissues. Take, for example, a potato, cut it into small pieces of about ½ inch square, and harden them in methylated spirit. Then embed in carrot and cut the sections, which should not be too thin. Stain in a 1 per cent. solution of methyl aniline violet, wash in water, and mount in glycerine jelly.

In mounting starch in glycerine jelly, care should be taken that the jelly is not too hot; if it be, the form of the starch will be altered.

Yeast.—Get some fresh baker's yeast, place a little of it in a bottle of sugar and water, and stand in a warm place for twenty-four hours. Pour off the sugar water, and add camphor water. Make a cell on a slide with black shellac cement, and let it dry; then apply a second coat of cement, and let this stand for a few minutes. Now take up some of the yeast in a glass tube and place a few drops in the cell; clean a cover-glass, and bring its edge in contact with the cement on one side of the cell; ease it down carefully, so that no air-bubbles may be enclosed; now press on the surface of the cover with a needle until it adheres firmly to the cell all round, drain off the excess of fluid, dry the slide with a clean cloth, and apply a coat of cement.

Mycetozoa or Myxomycetes.—Most of these fungi can be mounted in glycerine jelly after soaking in equal parts of rectified spirit and glycerine to remove the air, but in those forms which possess lime granules in the capillitium—a character of importance in classification—the calcareous matter disappears when in glycerine in any form. When this is the case, place the specimen in absolute alcohol until all air is removed, then transfer to clove oil, and mount in Canada balsam. Some specimens may, however, be rendered too transparent by the balsam; if so, mount them in a shallow cell in some neutral fluid such as camphor water.

In their ripe condition they may also be mounted dry as opaque objects.

Large fungi, such as Agaricus, should be hardened in methylated spirit for a week. Then place the desired portion in water,

and soak to remove spirit, transfer to gum and syrup, and when penetrated with the gum, freeze and make the sections with a Cathcart microtome, wash away all trace of gum with repeated changes of warm water, and mount unstained in glycerine jelly.

Preserving Fluid for Green Algæ.—Acetate of copper, 15 grains; camphor water, 8 ounces; glacial acetic acid, 20 drops; glycerine, 8 ounces; corrosive sublimate, 1 grain. Mix well together, filter, and keep in a stoppered bottle. The above fluid preserves the colour of chlorophyll for a long time; it may also be used as a mounting fluid. For very delicate specimens leave out the glycerine.

The specimens should be well washed in water; then pour off the water, and add a quantity of the copper solution.

To Mount in the Above.—For example, take Spirogyra as a filamentous alga. Make a cell with some black cement, and let it dry; then apply a second coat of cement, and allow this to nearly dry. Place some Spirogyra in the cell, and with needles separate the filaments; add a few drops of copper solution, and apply a cover-glass as directed for yeast.

Protococcus.—This can be obtained by scraping the bark of trees. Place it in a bottle of water, and let it stand for a few hours; now add a little copper solution—this will kill the specimens, and they will sink to the bottom of the bottle; pour off the water, and add more copper solution. Now make a cell as for Spirogyra; take up some of the protococci in a dipping-tube, and place them in a cell; wait a minute for the forms to settle on the bottom of the cell, and then apply a cover-glass; drain off the excess of fluid, dry the slides with a cloth, and apply a coat of cement.

Volvox, glœocapsa, desmids, etc., may all be preserved and mounted as above.

Antheridia and Archegonia of Mosses.—Place some male and female heads of mosses in methylated spirit for a few days, then transfer to equal parts of absolute alcohol and ether, in which they must be soaked for several hours. Pour off the alcohol and ether, and add a thin solution of celloidin, and soak for two or three days; then remove the stopper of the bottle, and let the celloidin evaporate to about half its original bulk. Now remove a specimen from the celloidin, and hold in a pair

of forceps until the celloidin sets, then place it in methylated spirit and soak for an hour or two to complete the hardening. The embedded specimen may now be fastened to a cork with a little celloidin, and longitudinal sections made in a Cathcart microtome, or it can be placed between two pieces of carrot, and the sections made with any ordinary well microtome. The sections must then be dehydrated in methylated spirit, cleared in oil of bergamot, and mounted in Canada balsam; or, if desired, they may be soaked in water to remove spirit, and be mounted in glycerine jelly.

Fertile Branch of Chara.—Chara is usually very dirty; to clean it, wash well in repeated changes of water, then in very dilute acid for a few minutes only; again wash in water, and preserve in camphor water.

Make a cell with shellac cement as directed above, place a fertile branch of Chara in it; and examine under a dissecting microscope or lens; with needles clear away the leaves from the archegonia and antheridia, fill the cell with camphor water, and apply a cover-glass.

When a deep cell is required for a specimen to be mounted in acetate of copper, never use one made of any metal. Vulcanite or glass cells must be used. To one side of a cell apply a coat of shellac cement and let it dry; now take a slide and warm it over a spirit-lamp; take up the cell in a pair of forceps, and bring the cemented side in contact with the centre of the warm slide, and press it down until it adheres firmly; then add another coat of cement to the upper side of the cell, and let it nearly dry, put in the specimen, fill the cell with solution, and apply the cover-glass.

Prothallus of Fern.—Preserve in acetate of copper and mount in the same fluid in a shallow cell.

Sporangia and Spores of Fern.—Place leaves of a fern with sporangia in methylated spirit for a few days to remove the air. Then soak in water for several hours. Warm a slide, and place a few drops of glycerine jelly on its surface, scrape off some sporangia, and place them in the jelly; now apply the cover-glass very carefully to avoid scattering the sporangia. The object is to keep them in a heap in the centre until the cover is flat; then press on the surface of the cover with the points of the forceps,

and, if possible, give the cover a little twisted motion. This will spread the specimens; it will also rupture some of the sporangia and let out the spores.

Isolating Antheridia and Oogonia from Fucus.—Take some conceptacles that have been hardened in methylated spirit, and make thick sections by hand only with a sharp knife. Place these in a strong solution of acid aniline green in spirit, and let them stand for two or three hours. Now place in water for a few minutes, and they will at once swell up like a mass of mucus. Place this on a slide and put another slide on top of it, press down the upper slide—this will squeeze out the contents of the conceptacles in little round masses. Separate the glasses, pick up one of the little lumps of antheridia or oogonia, place it in a few drops of glycerine jelly on a slide, then apply the cover-glass, which must be pressed down to spread the specimens.

Digestive Glands in Pitcher Plant.—Harden some strips of a pitcher in methylated spirit for a week. Then place in water and soak for a few hours. Then lay the tissue with the glandular surface next to the glass, and with a scalpel scrape away the outer wall. Now bleach the glandular portion in chlorinated soda, then wash well with water, stain in aqueous solution of acid aniline green, wash again in water to remove excess of colour, soak for several hours in dilute glycerine, and mount in glycerine jelly.

Aleurone.—Take the endosperm of a castor-oil seed, embed in carrot, place in microtome, and cut sections as thin as possible with a knife wetted with a little olive oil. As the sections are cut, put them on a slide, and place out of reach of dust until you are ready to mount them.

Make a shallow cell as directed with black enamel and let it dry, then proceed as directed for acetate of copper mounting, but use castor oil instead of copper solution. When the cover has become fixed, wash away the exuded oil with a soft brush and some turpentine, and, when dry, apply a good finishing coat of black enamel. Water and spirit are apt to injure the aleurone grains, so they should be avoided.

Marine Algæ.—The best place for collecting specimens is a rocky shore, and the most suitable time is when the tide is at its

lowest.   As a rule, the inshore weeds near high-water mark are green, lower down there is usually a belt of olive forms sheltering red plants beneath them, and where rocks overhang small shallow pools red forms also occur at this level.   At extreme low-water mark and beyond it are found brown tangles sheltering red plants again, while at the lowest depths the red weeds occur without shelter.   The specimens will be found by searching the rocks and pools, some will be growing on pebbles and on shells, others will be attached to rocks, and varieties may be found stranded on the shore, thrown there by waves, particularly after a storm, the tufts having been torn away and carried inshore from inaccessible regions.

For collecting, small tin boxes or an ordinary sponge bag will be found most suitable.   A strong chisel mounted on a stout stick will also be required for removing specimens from rocks that are out of reach.

Many specimens may be preserved in sea water for a considerable time, but, as a rule, the sooner they are mounted the better.

Mounting Process.—Remove the specimen from sea water and wash well in fresh water.   Place in a shallow white dish or saucer, select and cut off the portion that is to be mounted, and place it on a slide slightly warmed, drain away as much water as possible, and apply some glycerine jelly ; then, if necessary, lay or spread out the leaves or filaments with a needle and apply the cover-glass, allow the slide to cool, remove the excess of jelly around the edge of the cover, wash the slide in water, dry, and add several coats of enamel or varnish.

Corallines, whose tissues are hard and opaque, may be cleaned by soaking for a short time in a weak solution of hydrochloric acid, then wash well in water, and mount in glycerine jelly.

HARDENING AND PRESERVING ANIMAL TISSUES AND LISTS OF MATERIALS.

LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING, AND MOUNTING REAGENTS.

Tissues.	Hardening Reagent.	Staining Fluid.	Mounting Medium.	Animal.
Blood, human ...	Dry on slide	Eosin	C. Balsam	—
Ditto, amphibia ...	Ditto	Eosin and methyl green	Ditto	Frog.
Epithelium ...	2 per cent. bichromate potash	Picrocarmine	Farrant's	Ditto.
Endothelium ...	Nil	Silver nitrate	Farrant's or C. Balsam	Rabbit.
White fibrous tissue	Alcohol	Nil	Farrant's or glycerine jelly	Tail of rat.
Yellow elastic tissue	Chromic acid and spirit	Nil	Ditto	Lig. nuchæ of ox.
Adipose tissue ...	Methylated spirit	Hæmatoxylin	Ditto	Any animal
Tendon ...	Ditto	Ditto	Ditto	Sheep.
Adenoid tissue ...	Müller's fluid	Hæmatoxylin and eosin	C. Balsam	Cat.
Cartilage ...	Chromic acid and spirit	Ditto	Ditto	Ditto.
Bone ...	Chromic and nitric acid	Picrocarmine	Farrant's	Ditto.
Ditto, developing ...	Ditto	Hæmatoxylin and eosin	C. Balsam	Kitten.
Marrow ...	Methylated spirit	Hæmatoxylin	Ditto	Guinea-pig or cat.
Muscle, striated ...	2 per cent. bichromate potash	Hæmatoxylin and eosin	Ditto	Cat.
Ditto, non-striated...	Chromic acid and spirit	Hæmatoxylin	Balsam or Farrant's	Colon of rabbit.
Nerve-fibres ...	Osmic acid	Osmic acid	Farrant's	Sciatic of frog.
Ditto, trunk ...	Chromic acid and spirit	Hæmatoxylin and eosin	C. Balsam	Sciatic of cat.
Bloodvessels ...	Ditto	Ditto	Ditto	Cat.
Lymphatic glands ...	Müller' fluid	Ditto	Ditto	Ditto.
Tonsil ...	Methylated spirit	Ditto	Ditto	Ditto.
Thymus gland ...	Müller's fluid	Ditto	Ditto	Human fœtus or calf.

LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING, AND MOUNTING REAGENTS—*Continued.*

Tissues.	Hardening Reagent.	Staining Fluid.	Mounting Medium.	Animal.
Skin ...	Methylated spirit	Hæmatoxylin and eosin	C. Balsam	Human palm of hand.
Nail ...	Ditto	Ditto	Ditto	Human fœtus.
Scalp ...	Ditto	Ditto	Ditto	Ditto.
Heart muscle ...	Chromic acid and spirit	Ditto	Ditto	Cat.
Trachea ...	Ditto	Ditto	Ditto	Ditto.
Lung ...	Ditto	Ditto	Ditto	Ditto.
Tooth ...	Chromic and nitric acid	Picrocarmine	Farrant's	Ditto.
Ditto, developing ...	Ditto	Ditto	Ditto	Kitten about two months old.
Tongue ...	Chromic acid and spirit	Hæmatoxylin and eosin	C. Balsam	Cat.
Œsophagus ...	Ditto	Ditto	Ditto	Ditto.
Stomach, cardiac end	Absolute alcohol	Soluble aniline blue	Ditto	Ditto.
Ditto, pyloric end ..	Ditto	Hæmatoxylin and eosin	Ditto	Ditto.
Small intestine ...	Chromic acid and spirit	Ditto	Ditto	Ditto.
Large intestine ...	Ditto	Ditto	Ditto	Ditto.
Liver ...	2 per cent. bichromate potash	Ditto	Ditto	Ditto.
Pancreas ...	Absolute alcohol	Ditto	Ditto	Ditto.
Salivary glands ...	Ditto	Ditto	Ditto	Ditto.
Spleen ...	2 per cent. bichromate potash	Ditto	Ditto	Ditto.
Suprarenal glands ...	Methylated spirit	Ditto	Ditto	Guinea-pig or cat.
Thyroid glands ...	Ditto	Ditto	Ditto	Cat.
Kidney ...	2 per cent. bichromate potash	Ditto	Ditto	Ditto.
Ureter ...	Chromic acid and spirit	Ditto	Ditto	Ditto.
Testicle ...	Methylated spirit	Ditto	Ditto	Ditto.

LIST OF TISSUES AND ORGANS, AND THE MOST SUITABLE HARDENING, STAINING, AND MOUNTING REAGENTS—*Continued.*

Tissues.	Hardening Reagent.	Staining Fluid.	Mounting Medium.	Animal.
Vas deferens ...	Methylated spirit	Hæmatoxylin and eosin	C. Balsam	Cat.
Epididymis ...	Ditto	Ditto	Ditto	Ditto.
Prostate ...	Ditto	Ditto	Ditto	Ditto.
Ovary ...	Ditto	Ditto	Ditto	Ditto.
Fallopian tube ...	Ditto	Ditto	Ditto	Ditto.
Uterus ...	Ditto	Ditto	Ditto	Ditto.
Mammary gland ...	Ditto	Picrocarmine	Farrant's or C. Balsam	Ditto.
Spinal cord ...	2 per cent. bichromate ammonium	Aniline blue-black	C. Balsam	Ditto.
Medulla oblongata...	Ditto	Ditto	Ditto	Ditto.
Pons Varolii ...	Ditto	Ditto	Ditto	Ditto.
Cerebellum ...	Ditto	Ditto	Ditto	Ditto.
Cerebrum ...	Ditto	Ditto	Ditto	Ditto.
Eyelid ...	Methylated spirit	Hæmatoxylin and eosin	Ditto	Cat or human fœtus.
Cornea ...	Müller's fluid	Ditto	Ditto	Cat or sheep.
Choroid ...	Ditto	Ditto	Ditto	Sheep.
Crystalline lens ...	2 per cent. bichromate potash	Picrocarmine	Farrant's	Cat.
Retina ...	Müller's fluid	Hæmatoxylin and eosin	C. Balsam	Ox.
Sclerotic ...	Ditto	Ditto	Ditto	Ditto.
Optic nerve ...	Ditto	Ditto	Ditto	Ditto.
Olfactory mucous membrane	Ditto	Ditto	Ditto	Rat.
Internal ear. Coch-lea	Müller's fluid, and de-calcify	Carmine in bulk	Ditto	Guinea-pig.

LIST OF BOTANICAL SPECIMENS, AND THE MOST SUITABLE PRESERVING, STAINING, AND MOUNTING MEDIA.

Specimen.	Preserving Reagent.	Staining Fluid.	Mounting Medium.
Stems, young	Methylated spirit	Hæmatoxylin	C. Balsam.
Ditto, older	Ditto	Carmine and acid green	Ditto.
Leaves	Ditto	Hæmatoxylin	Ditto.
Ovaries	Ditto	Ditto	Ditto.
Anthers	Ditto	Borax carmine	Ditto.
Epidermis for stomata	Macerate in water	Methyl aniline	Glycerine jelly.
Fibro-vascular tissues	Ditto	Acid aniline green	Ditto.
Yeast	Camphor-water	Unstained	Camphor-water.
Green algæ	Acetate of copper solution	Ditto	Acetate of copper solution.
Red algæ	Dilute methylated spirit	Ditto	Glycerine jelly.
Protococcus	Acetate of copper solution	Ditto	Acetate of copper solution.
Volvox	Ditto	Ditto	Ditto.
Desmids	Ditto	Ditto	Ditto.
Raphides	Macerate in water	Ditto	C. Balsam.
Starches	Methylated spirit	Ditto	Glycerine jelly.
Fertile branch of chara	Ditto	Ditto	Camphor-water.
Antheridia and archegonia of mosses	Ditto	Ditto	C. Balsam.

THE MICROSCOPE IN GEOLOGY

An Introduction to the Use of the Petrological Microscope.

By Professor FREDERIC J. CHESHIRE, C.B.E., F.Inst.P.,
President of the Royal Microscopical Society.

The petrological microscope is nothing more than an ordinary microscope fitted with certain optical and mechanical adjuncts —as will be seen on reference to Fig. 96, p. 179—by the proper employment of which a more or less complete quantitative and qualitative determination can be made of the optical properties of transparent crystals, as exhibited in polarized light, and thus the identification of such crystals either effected or facilitated. An elementary knowledge at least of the explanation of polarization phenomena is, therefore, imperative for the intelligent and efficient use of the microscope in question—a fact which is too often overlooked by the microscopist.

Polarization of Light.—It is a very curious fact that the understanding of polarization phenomena, when associated with light-waves, should present so many difficulties, when it is remembered that polarized waves are the only forms of wave-motion with which most people are made familiar by their everyday experiences. A pebble dropped into a pond produces a number of small waves, which, starting at the point where the pebble fell into the water, spread outwards in all directions in the form of ever-increasing circles, until they have passed over the entire surface of the pond. But, although these waves travel over the surface, we know that the particles of water concerned in their production do not travel, but simply move up and down. A floating cork, for example, is not carried forward by these waves, but simply rises and falls as each wave passes beneath it.

PLATE V.

INTERFERENCE FIGURES OF ARRAGONITE. (HAUSEWALDT.)

As seen in convergent sodium light between crossed nicols.

FIG. 82.   Section ½ mm. thick.   FIG. 83.

FIG. 84.   Section 4 mm. thick.   FIG. 85.
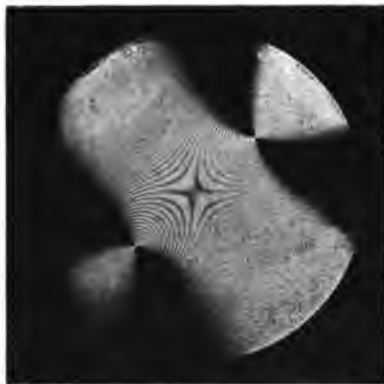
Figs. 82 and 84 with extinction directions of crystal parallel to those of nicol.
Figs. 83 and 85 with extinction directions of crystal diagonal to those of nicol.

Here, then, we have a case in which waves are propagated from one place to another by the vibratory motion of a large number of particles in one plane only. When waves are transmitted in this way by the motions of particles in a single and constant plane, the wave is said to be polarized. But a simpler and even more instructive example might have been given.

Let us suppose that a long rope is stretched rather loosely between two boys, one of whom, by a rhythmic movement of his hand in an up-and-down direction, produces waves in the rope which pass continuously to the other boy. In this case, too, since the vibrations of the particles of which the rope is made up take place in one constant plane only, the resulting waves
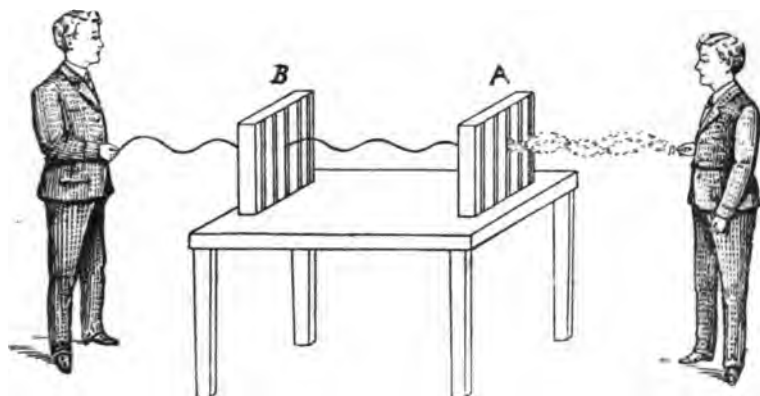
FIG. 86.—ROPE POLARISCOPE: NICOLS PARALLEL.

are polarized ones. But now, as in Fig. 86, let the rope be passed through two gratings, A and B—such, for example, as those used for covering street gullies—in both of which the bars are at first arranged vertically. Now let the first boy start waves along the rope—not by confining the motion of his hand to one plane only, but by changing the direction both rapidly and arbitrarily, so that a jumble of waves is sent along the rope in which the vibrations of the particles take place more or less in all directions. It is clear that these waves are no longer polarized, since the various particles concerned are moving in different planes. This state of affairs would only exist, however, between the first boy and the grating A, for since the bars of the latter are vertical, it is clear that each wave as it falls upon the grating

would, in general, be partly stopped and partly transmitted; and, since in the motion transmitted the vibrations would take place in one constant plane only, it would be polarized. The grating A would thus act as a polarizer, and we should have a succession of polarized waves passing from it to the second grating B, and thence, since the bars of B are also arranged vertically, to the second boy. Had the bars of the grating B been arranged horizontally—that is, at right angles to those of the first—it is clear that the polarized waves falling upon the second grating would be stopped as in Fig. 87, but in all intermediate positions of these bars, between the vertical and
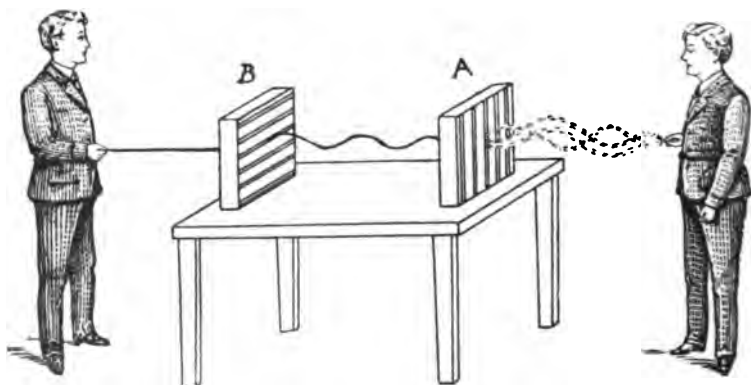
FIG. 87.—ROPE POLARISCOPE: NICOLS CROSSED.

horizontal, the waves from A would be partly stopped and partly transmitted with the plane of vibration changed. Finally, were it required to determine the plane in which the vibrations were taking place in the waves falling upon B, it would only be necessary to rotate this grating into such a position that no waves were transmitted. The bars would then be at right angles to the sought-for direction. In this way the grating B could be used to test the polarized condition of the waves falling upon it —i.e., as an analyzer.

Polarized Light.—Ordinary light may be looked upon as consisting of waves transmitted through the ether of space by the to-and-fro motions of the particles of that ether in all directions across the line of march of the waves; but when such a beam of light falls upon a nicol prism, it is polarized—that is,

after passing through the prism, the light-waves are found to have their vibrations in a single and constant plane. The nicol prism, therefore, under these circumstances acts upon light-waves in an analogous way to that in which the grating A acted upon the rope-waves. Similarly, the polarized light produced by the action of one nicol prism being allowed to fall upon a second one similarly arranged, will pass through it, just as the waves from the grating A passed through the grating B, when similarly arranged, as in Fig. 86. The second nicol also being turned through a right angle from the position just described, completely stops the light falling upon it from the first one, just as the second grating B stopped the waves from A in the position shown by Fig. 87. Finally, in all intermediate positions of the second nicol the light will be more or less completely transmitted, but with its plane of vibration changed. To sum up, therefore, we may say that—

1. In ordinary or common light, such as sunlight, the vibrations of the ether particles concerned in the transmission of the light-waves take place in every possible direction across the direction in which the light is moving.

2. A nicol prism (or its equivalent) is a kind of optical grating which reduces the vibrations of ordinary incident light to a single direction or plane only, and is thus said to act as a polarizer.*

3. A nicol prism, when polarized light falls upon it, transmits it in general more or less completely with its plane of vibration changed, but in the particular case when it is so arranged that the direction in which it allows ether vibrations to take place is at right angles to the direction in which the vibrations in the incident polarized light are taking place, it stops the incident light altogether, and thus acts as an analyzer.

Double Refraction.—The peculiar optical property possessed by most crystals in virtue of which their examination and differentiation in a petrological microscope becomes possible is known as double refraction, and is due to what might very appropriately be called 'optical grain.' A piece of wood has a

* This action of a nicol prism must not be confounded with that of the grating employed in spectrum analysis, which acts, of course, in an entirely different way.

grain which is usually, it is true, apparent to the eye; but even in the absence of such evidence the fact could soon be determined experimentally, as by attempting to split the wood with a hatchet in various directions. Rock crystal, the clear and transparent variety of quartz, sometimes takes the form of long prisms of hexagonal cross-section, as shown by Fig. 88. Now, it is found that if a slice be cut lengthwise from such a crystal, as indicated at AA, and smeared with wax on one of its faces, the application of heat to a point on that face will cause the wax to melt. The area over which the melting occurs will not, however—as in the case of glass, for example—take a circular form, but an elliptical one, with the major axis of the ellipse parallel to the geometrical axis of the crystal. This experiment shows that heat is transmitted more rapidly along the crystal than across it. In a slice cut across the axis, as at BB, the melted area takes a circular form, showing that across the crystal heat is transmitted equally in all directions. Rock crystal is thus shown to be possessed of a kind of grain running in the direction of its length. In consequence of this grain, which is optical as well as thermal, it is found that if a beam of light be allowed to fall normally upon the face of such a slice of rock crystal, the latter will be found to act as a kind of double grating, sifting and transmitting the incident light as two beams, in one of which the vibrations take place only in the direction of the length of the crystal, and in the other beam across it only. A nicol prism, it may be remarked, is a crystal of Iceland spar in which one of the two beams just referred to is thrown out of the way by reflection on an artificial interface. A beam of light passed along the axis of a quartz crystal is not split up into two beams, but in any other direction it is; hence rock crystal is what is known as a uniaxial crystal. In other crystals, as mica and selenite, there are two directions in which light passes without being split up; these are therefore known as biaxial crystals.

FIG. 88.—CRYSTALS OF QUARTZ.

Now the two polarized beams into which light is, in general, split up on its passage through a crystal, travel with different speeds, and in most cases in slightly different directions—hence the term 'double refracting.' In the case of rock crystal, for example, the beam in which the vibrations occur along the axis of the crystal travels more slowly than the beam in which the vibrations occur across it.* Let such a slice of rock crystal be placed between crossed nicols, and arranged with its axis inclined to the vibration-directions P and A of the polarizer and analyzer respectively, as shown by Fig. 89. Then polarized light coming upwards from the polarizer towards the observer will be resolved by the crystal into two beams with vibrations in rectangular planes and passing with different speeds. These two beams, falling upon the analyzer, will each again be resolved into two beams—one with horizontal and the other with vertical vibrations. The first of these will pass the analyzer, the second will be stopped. But, further than this, the light which passes the analyzer and emerges as a single polarized beam is com-
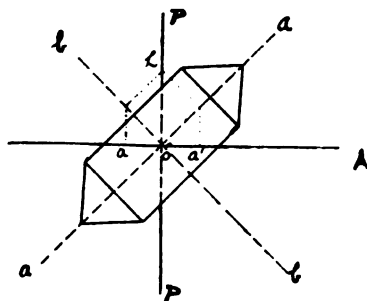
FIG. 89.—SECTION OF QUARTZ CUT PARALLEL TO AXIS.

pounded of two beams of equal intensities, one of which passed through the crystal with vibrations along *aa*, and the other with vibrations along *bb* ; and, since these beams travelled at different speeds, it follows that upon being compounded by an analyzer the waves in one will be more or less out of step with those in the other, with the result that interference will take place, and the waves corresponding to any colour transmitted in opposite phase by the two paths will be destroyed, leaving the transmitted beam coloured. Thus, when the crystal is of such a thickness that the waves by the slow path emerge 550 micro-

* This statement is made upon the usual assumption that the vibrations of the ether take place in a direction at right angles to the plane of polarization. It should also be remembered that light polarized by reflection at a plane glass surface is *defined* as being polarized in the plane of reflection. It follows, therefore, from the given assumption and the definition, that the vibrations in the polarized reflected light are executed in directions parallel to the surface of the glass.

millimetres (550 $\mu\mu$) behind those passing by the fast path, green light with a wave-length equal to this quantity will be cut out, leaving the transmitted beam of the complementary colour red. As shown by Fig. 89, an amplitude *oh* in the polarizer is, after being resolved along each of the two vibration-directions in the crystal and the vibration-direction (horizontal) of the analyzer, represented by two equal amplitudes *oa* and *oa'*. Different colours produced in this and analogous ways have been very carefully studied, and are set out in the following table. This succession of colours would be produced by a wedge of selenite with its vibration-directions adjusted diagonally as in Fig. 89, between crossed nicols, the thickness of the wedge increasing from nothing up to about 0·2 mm. The first column gives the retardation—*i.e.*, the distance in micromillimetres which one beam emerges behind the other after passing through the crystal—whilst the second column gives the colour corresponding to such retardation.

NEWTON'S COLOUR SCALE ACCORDING TO QUINCKE.

Retardation in Micro- millimetres.	Interference Colour between Crossed Nicols.	Order.	Retardation in Micro- Millimetres.	Interference Colour between Crossed Nicols.	Order.
0	Black	First.	848	Yellowish-green	Second.
40	Iron-grey		866	Greenish-yellow	
97	Lavender-grey		910	Pure yellow	
158	Greyish-blue		948	Orange	
218	Clearer grey		998	Bright orange-red	
234	Greenish-white		1,101	Dark violet-red	
259	Almost pure white				
267	Yellowish-white		1,128	Light bluish-violet	Third.
275	Pale straw-yellow		1,151	Indigo	
281	Straw-yellow		1,258	Greenish-blue	
306	Light yellow		1,334	Sea-green	
332	Bright yellow		1,376	Brilliant green	
430	Brownish-yellow		1,426	Greenish-yellow	
505	Reddish-orange		1,495	Flesh colour	
536	Red		1,534	Carmine-red	
551	Deep red		1,621	Dull purple	
565	Purple	Second.	1,652	Violet-grey	Fourth.
575	Violet		1,682	Greyish-blue	
589	Indigo		1,711	Dull sea-green	
664	Blue (sky-blue)		1,744	Bluish-green	
728	Greenish-blue		1,811	Light green	
747	Green		1,927	Light greenish-grey	
826	Lighter green		2,007	Whitish-grey	

Rotary Polarization.—Certain crystals possess in a certain direction the remarkable power of rotating or twisting the plane of vibration of a polarized beam passing through them. Thus, if a slice of rock crystal 1 mm. thick, cut from the crystal normal to the axis as at BB, Fig. 90, be placed between crossed nicols in white light, it is found that the analyzer no longer stops the light, and that no position can be found for it in which it does stop it. In the case of sodium light, however, it is found that a rotation of the analyzer from its crossed position with respect to the polarizer through an angle of 22° again establishes darkness. In some instances this necessary rotation has to be made in

the direction of the hands of a clock, from the observer's point of view, about the direction in which the light is passing to the eye, whilst in others it has to be made in the opposite direction. In the first case the rotary polarization is said to be right-handed, and in the second case left-handed. If, then, a beam of polarized sodium light, in which the vibrations are vertical, is allowed to pass along the axis of a quartz crystal, the plane of vibration will not remain vertical,
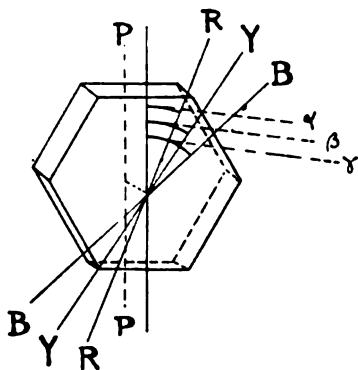
FIG. 90. — SECTION OF QUARTZ, NORMAL TO AXIS, TO SHOW ROTARY POLARIZATION.

but will be gradually rotated or twisted about that axis at the rate of 22° per mm. of length of the crystal. For different colours in the incident polarized white light, the rate of turning is different. Red light, for example, has its plane of vibration twisted at the rate of 13° per mm., whilst blue, on the other hand, is twisted through as many as 33° in the same length. Thus, if Fig. 90 be taken to represent this action in a slice of right-handed quartz of the specified thickness in incident white light, polarized with its vibrations along the line PP, and passing upwards to the eye, the vibration planes for the red, yellow, and blue will be twisted into the directions RR, YY, and BB respectively, through angles a, β, and γ, equal to 13°, 22°, and 33° respectively. The analyzer, therefore, set originally parallel

with the polarizer and rotated in the direction of the hands of a clock, would allow in succession the colours red, yellow, and blue to pass in predominance to the eye, so that the crystal would appear to change in colour during the rotation of the analyzer.

Optical Adjuncts for the Petrological Microscope.

Crystallographic determinations are very much facilitated by the employment of a number of optical adjuncts, the more indispensable of which are set out below.

Mica Quarter-Wave Plate.—This consists of a cleavage plate of mica of such a thickness that sodium light, with a wavelength of 589 $\mu\mu$, in passing through it, along one of the two vibration-directions possible in a double-refracting crystal, emerges a quarter of a wave-length behind that passing through by the other rectangular vibration-direction. These vibration-directions are often, therefore, referred to as the 'fast' and 'slow' directions to differentiate them. If a quarter-wave mica then be placed on a crystal section, so that similar directions in the two sections are parallel to one another, the effect is the same as that which would have been obtained by increasing the thickness of the crystal section, and if the latter should be between crossed nicols with its vibration-direction inclined at 45° to the vibration-directions of the nicols, its colour would rise in Newton's scale—*i.e.*, correspond to a greater retardation. By superposing the mica with its fast direction parallel to the slow direction of the crystal section, the effect would be the same as that which would have been obtained by decreasing the thickness of the crystal section, so that in this case the colour would descend in Newton's scale—*i.e.*, correspond to a less retardation. By the use of a quarter-wave mica in this way the fast and slow directions of crystal sections are differentiated.

Selenite or Gypsum Plate.—When it is required to differentiate the fast and slow directions of a crystal section with small bi-refracting power, a selenite plate of such a thickness as to give a rose colour between crossed nicols is employed. Superposed upon a crystal section with similar directions parallel, the colour changes to blue, whilst the placing of fast on slow changes the colour to red.

Klein Quartz Plate.—A plate of quartz 3·75 mm. thick, and cut at right angles to the axis of the crystal, gives between crossed nicols, and in virtue of its rotary polarizing power, a purple colour. In this case the orientation of the plate does not affect either the intensity or the colour of the light transmitted.

Bertrand Plate.—This plate is made up of four quadrantal sectors of alternately right- and left-hand quartz, cut at right angles to the axis of the crystal, and 2·5 mm. thick.
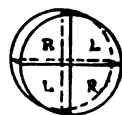
Fig. 91.
BERTRAND
PLATE.

Fedorow Mica-Steps. — This is built up by superposing some sixteen strips of quarter-wave mica, all with similar directions parallel, in such a way that each strip is about 2 mm. shorter than the one immediately below it. Sixteen steps are thus formed, which effect in succession retardations,
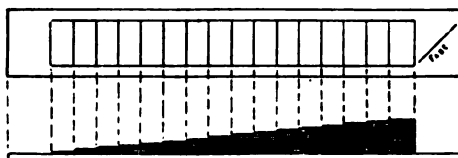
FIG. 92.—MICA-STEPS.

increasing by a quarter-wave at each step, commencing with a quarter-wave, and finishing with four waves.

Quartz Wedge.—A thin slice of quartz cut parallel to the crystallographic axis is ground into the form of a thin wedge. Could such a wedge be ground to an infinitely thin edge, it would give at this edge, when oriented with its vibration-directions in diagonal adjustment between crossed nicols, the black of Newton's colour scale, followed by all the colours of the scale in ascending order in passing along the wedge to the thick end. Wedges giving the first six orders of Newton's scale, or some smaller number if required, are thus made. To avoid the necessity for grinding a very thin edge, the quartz plate A from which the wedge is to be made is cemented to a thin plate of selenite B, with the fast direction of one parallel to the slow direction of the other. By this device it is made easy to get the starting black at the thin end of the quartz without reducing that end to a less thickness than the selenite foundation-plate

possesses.   Sometimes this foundation-plate is made to give
the sensitive rose tint of the first or second order, and is made
to project for a short distance beyond the thin end of the quartz.
The wedge should carry a scale along its length, from which the
retardation in micromillimetres at any point of the wedge, and
for any given tint, can be determined.

It will be found that the optical adjuncts referred to above,
as produced by different makers, are unfortunately not uniformly
mounted as regards the orientation of their vibration-directions
with respect to the length of the plate or wedge.   Sometimes
the fast direction is coincident with the length of the plate,
sometimes it is across it, and sometimes it will be found inclined
at 45° to the length.   The last disposition has the advantage
that by inverting the plate in the cross slot in the tube of the
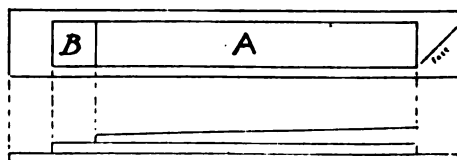
FIG. 93.— QUARTZ WEDGE.

microscope, the optical superposition of fast on fast can be
changed for slow on fast without any difficulty.

The Construction of the Petrological Microscope.—Fig. 96
shows a first-class modern instrument.   The sub-stage polarizer
is associated with a condensing system for convergent light, the
top lens of which can be turned to one side when plane-polarized
light is required.   The stage is rotatable, and graduated to read
to 5′ with the help of a vernier.   The collar to which the objective
is secured is fitted with centring screws, and is made with a slot
into which the usual mica and gypsum compensators can be
introduced.   The analyzer fitted above the objective can be
pushed radially into and out of action.   The Bertrand lens slides
into position near the middle of the length of the tube, and an
auxiliary analyzer with divided circle and a slot for compensators
may be fitted over the eyepiece.   The fine adjustment head is
graduated to read directly to a thousandth of a millimetre.

Figs. 94 and 95 show the analyzer and polarizer as mounted
for use with an ordinary microscope.

FIG. 94.

FIG. 95.
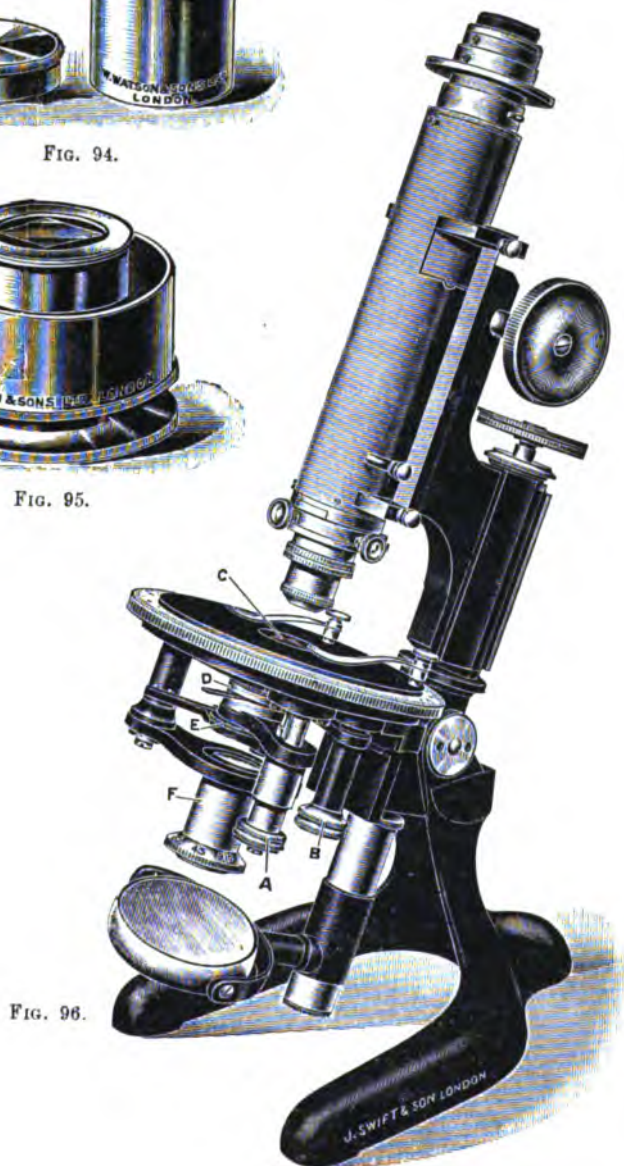
FIG. 96.

Preliminary Adjustment of a Petrological Microscope.— Before any work is done the following adjustments should be carefully tested, and, if necessary, made:

1. Centring of the objective.
2. Rectangularity of the cross-wires in the eyepiece.
3. Rectangularity of the vibration planes of the two nicol prisms.
4. Parallelism of the cross-wires to the vibration planes of the two nicol prisms when the latter are crossed.

Centring of the Objective.—This is done in microscopes of the usual type by the manipulation of two radial set-screws acting against the collar in which the objective is secured. A slide should be placed upon the stage, and a prominent point or feature of it adjusted to the intersection of the cross-wires. Upon a complete rotation of the stage the point selected will describe a small circle in the field of view; consequently, when the stage has been rotated through 180° only, the point selected will have its maximum displacement from the intersection of the cross-wires. Stop the rotation, therefore, at this point, and turn the adjusting screws so as to move apparently the intersection of the wires half-way towards the selected point. The adjustment will now be found to be very nearly correct. Repeat the operation until it is quite so.

Rectangularity of the Cross-Wires in the Eyepiece.— Place a slide with a fine straight line ruled upon it on the stage, and adjust it until the projected image of the line coincides with one of the cross-wires. Note the angular position of the stage, and rotate it carefully through a right angle. The projected image of the line should now be parallel to the second cross-wire. If the centring of the objective has been first effected as above, the projected image in the second case will coincide with the second cross-wire.

Rectangularity of the Vibration Planes of the Nicol Prisms.—Darkness of the field is not a sufficiently delicate test for this adjustment, but, if the necessary adjunct—a Bertrand quarter-quartz plate—is not available for a more delicate adjustment, darkness should be obtained a number of times by rotation of the polarizer alternately in opposite directions. The mean of the various readings should be taken as the true one.

To obtain a better result, place a Bertrand plate upon the stage, set the polarizer to zero, and slide the analyzer into position. If the vibration planes of the two nicols are accurately at right angles to one another, the quadrants of the Bertrand plate will appear to have the same tint. Otherwise the colours of adjacent quadrants will not match, in which event the polarizer must be rotated until they do match, when the necessary zero correction should be read off on the polarizer. If the polarizer is not fully graduated so as to allow of this being done, a fine vertical line should be drawn across the junction of the polarizer mount and the sleeve into which it is pushed. Better still, if possible, the polarizing prism should be rotated in its mount, until the latter being at zero, the adjustment is correct.

Parallelism of the Cross-Wires to the Vibration Planes of the Crossed Nicols.—A needle-shaped crystal such as anhydrite (anhydrous sulphate of calcium, crystallizing in the orthorhombic system), in which one of the directions of extinction is parallel to the long edges of the crystal, should be placed on the stage between crossed nicols, and rotated until extinction is obtained. Pull the analyzer out, when the crystal should be seen ranged parallel to one of the cross-wires. Turn the crystal over on the stage and repeat.

Examination and Identification of the Crystalline Constituents of Rock Sections.

As this chapter does not profess to be anything more than an introduction to the use of the petrological microscope, no attempt will be made to describe the complete and systematic examination usually made of crystal sections by expert mineralogists. Some of the simpler determinations only will be indicated. Suppose that an angular crystal which lights up and darkens between crossed nicols upon rotation of the stage is to be examined, we could proceed to determine (1) the angles between the sides of the crystal; (2) the angular positions of the extinction-directions with respect to the sides ; (3) the differentiation of these extinction-directions into fast and slow ; and (4) the retardation of the section.

To Measure the Plane Angles of a Crystal Section.— Neither nicol is necessary. Adjust the section on the stage

until one of the sides of the section is projected along a cross-wire. Take the stage (angular) reading. Rotate the stage until the second side is brought into alignment with the same cross-wire. Take a second stage reading. The difference between these two readings gives the desired angle.

To Determine the Angular Positions of the Extinction-Directions.—Cross the nicols and adjust the section until a side of the crystal coincides with a cross-wire. Take the stage reading. Rotate the stage to extinction. Take the stage reading again. The difference is the desired angle. Repeat, and take the mean value of the results. To make a more accurate determination, advantage is taken of the fact that the four sectors of a Bertrand quarter-quartz plate, placed in the eyepiece between accurately crossed nicols, will appear of one uniform tint whenever a bi-refracting plate on the stage is rotated into such a position that its vibration-directions are parallel to those of the crossed nicols.

This method necessitates the employment of an analyzer above the eyepiece.

To Differentiate the Extinction-Directions.—This may be done by the use of a mica quarter-wave plate in the way already described. When, however, the bi-refracting power of the section being examined is very small, it is better to employ a sensitive selenite plate (so-called red of the first order). When this is introduced into the cross-slot just over the objective, so that its fast direction is parallel to the fast direction of the crystal section, the rose colour changes to a blue ; whilst when the fast direction is superposed on the slow of the section, the colour becomes a bright red.

Retardation.—This quantity is most simply determined by the use of the quartz wedge or the mica-step compensator. Unfortunately, however, these cannot be used satisfactorily in the usual slot over the objective, because in neither case, in the final adjustment, is one thickness only of the compensator operative. In the mica-step, for example, two or more steps must be interposed in the path of the light rays proceeding from the objective to form the image in the eyepiece, whilst in the case of the quartz wedge quite an appreciable fraction of the total length must be interposed. Further, in the latter case,

the retardation scale cannot be used since it is not in focus.
When a very low-power objective is sufficient, the compensators
can be superposed on the section on the stage, and the retarda-
tion determined directly by the position, in the case of the
quartz wedge, of the black band on the retardation scale when
the fast direction of the wedge is superposed on the slow direc-
tion of the section. In the case of the mica-step the retardation
may be equal to that of an integral number of steps, but more
generally it falls between two of these, and an estimate of its
value has to be made. These compensators should always, if
possible, be used in the focal plane of the eyepiece. In that
event, of course, the usual analyzer must be thrown out of action
and one placed instead over the eyepiece.
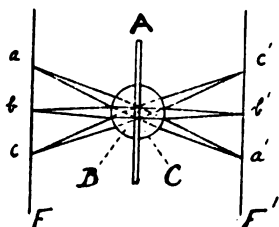
Examination in Convergent Light.—To understand the
optical action which is taking place in the microscope when it
is being employed for the examination of sections in convergent
light, it will be better to consider first the simple case (Fig. 97),
in which a plate of bi-refracting crystal A cut at right angles to
the axis—a plate of calcite, say—is interposed between two nearly
hemispherical lenses, B and C. Further, let plane-polarized
light, no matter how produced, start from the point *b*, on the
axis of the system and in the principal focal plane F of the
lens B, and passing through the lens B, plate A, and lens C,
be brought to a focus by the latter at the point *b′* in the focal
plane F′ of the lens C. Light from points *a* and *c* will similarly
be brought to a focus in points *a′* and *c′* respectively. Now, it
will be observed that in each of these three cases the light that
actually passes through the plate A is in the form of parallel
rays, but that the inclination which any particular bundle of
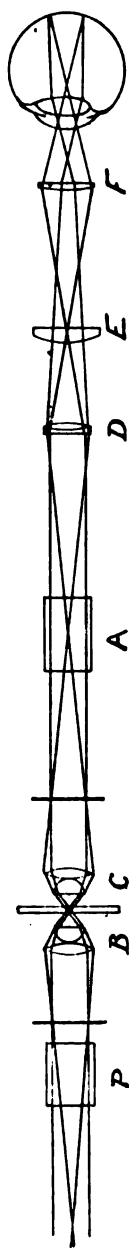
parallel rays makes with the axis of the crystal —the line bb'—depends upon the distance bc or ba. In the plane F', therefore, it follows that the light falling in the circular line struck with a radius $b'c'$, around the point b', is light, the whole of which has passed through the crystal plate at the same angle of inclination to the axis. In the plane F', therefore, we get the familiar interference figure which, when looked at through a crossed nicol, appears as a number of concentric rainbow-tinted rings, with a black cross marking them off into quadrants.

In the actual microscope the condenser B, Fig. 98, functions as the lens B of Fig. 97, and the objective C as the lens C of Fig. 97. The interference image shown as being focussed in the upper focal plane of the objective is projected by the Bertrand lens D and the field-lens E of the eyepiece, into the stop-plane of the latter, and again by the eye-lens F on to the retina of the eye of the observer. The analyzer is shown fitted between the Bertrand lens and the objective. The eyepiece and the Bertrand lens thus act together as a low-power compound microscope to magnify the figure in the upper focal plane of the objective C. In the absence of the Bertrand lens the eyepiece projects the interference figure into the Ramsden circle, where it can be very satisfactorily observed with a powerful pocket magnifier. In the latter case a small stop may be placed in the stop-plane of the eyepiece to cut off all light except that which has passed through the crystal under examination.

Plate V., from the atlas of the late Dr. Hausewaldt, shows the interference figures in convergent sodium light and between crossed nicols of sections of arragonite—the first pair due to a specimen ½ mm. thick, the second pair due to one of 4 mm. thickness.

Figs. 82 and 84 show the figures when the extinction-directions of the crystal are adjusted parallel

Fig. 98.—Ray-Diagram for a Microscope arranged for Convergent Light.

to those of the nicol; Figs. 83 and 85 when those directions are adjusted diagonally.

The attention of the reader desirous of further information is directed to a paper by Dr. John Evans in the Proceedings of the Geologists' Association, vol. xxi., part 2, 1909, on 'The Systematic Examination of a Thin Section of a Crystal with an Ordinary Petrological Microscope.' It is to be regretted that this invaluable brochure has not been published in a more accessible form. The following textbooks may also be referred to—viz., 'Anleitung zum Gebrauch des Polarisationsmikroskops,' by Weinschenk, Freiburg im Breisgau, 1906; 'Traité de Technique Minéralogique et Petrographique,' by Duparc and Pearce, Leipzig, 1909; and 'Manual of Petrographic Methods,' by A. Johannsen (Second Edition), London, 1918. The last book is strongly recommended for use by the advanced student.

THE MICROSCOPE IN ENGINEERING

By F. IAN G. RAWLINS, F.R.M.S.

As long ago as the year 1665 Dr. Hooke published in his 'Micrographia' an account of an investigation which he had made on the edge of a razor, using for this purpose a primitive form of microscope, in which he placed small globules of glass, and by means of which he intended to increase considerably the low magnifications usually obtainable in his time.

The result was that, in spite of the tremendous distortion brought about by the use of such an imperfect optical system, he was able to show that the razor edge, apparently so smooth and sharp, appeared under his microscope—to quote his own words—as 'full of ridges and furrows.'

The march of two and a half centuries finds engineers and metallurgists engaged on a quest not essentially different from that to which Hooke had devoted his attention ; but the means at disposal and the problems to be elucidated are now enormously increased, and a description of both of these is the primary purpose of this article. It may be well to point out the close connection between metallurgy and its allied occupations of metal casting and founding on the one hand, and engineering, with its employment of the material in a finished state, on the other. Safety, economy, and efficiency in general depend, to no small extent, upon the thoroughness with which metals and alloys are tested, and whether or not they are really suitable for their purpose.

The employment of the microscope in engineering has now become almost a necessity, for the complexity of the materials in use is so great that, without some such aid, accurate control

PLATE VI.
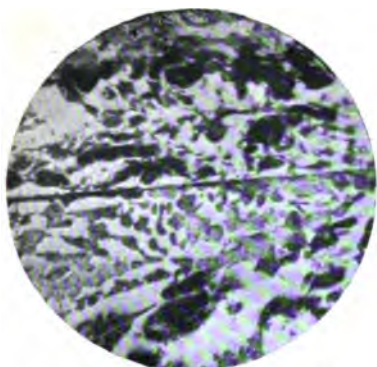
FIG. 99.—SPIEGELEISEN. × 70.
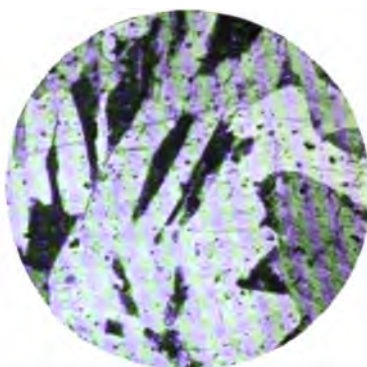
FIG. 100.—MILD STEEL. × 50.
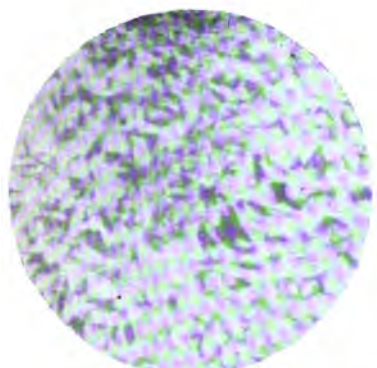
FIG. 101.—ANNEALED STEEL. × 70.

FIG. 102.—IRON-ZINC ALLOY. × 60.
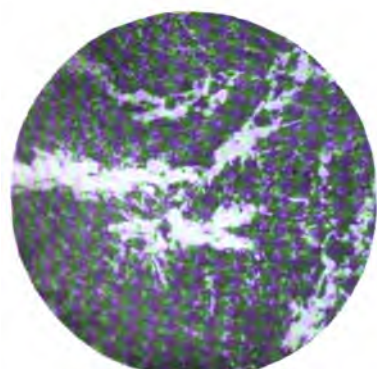
FIG. 103.—PEARLITE (DARK) AND
CEMENTITE (LIGHT). × 60.
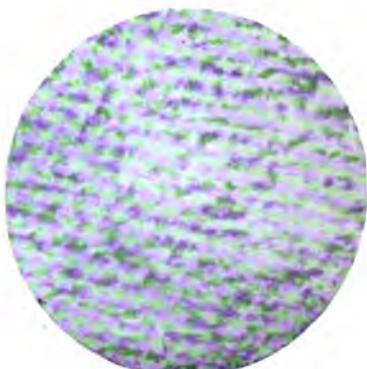
FIG. 104.—STEEL FATIGUED. × 70.

both in manufacture and in use is out of the question. Let it be made clear, before proceeding further, that the great advantage gained by microscopy over other methods of analysis is that the material examined is not destroyed, and that imperfections, weaknesses, or other sources of trouble, are laid bare *in situ ;* and that once a cause of failure has been located, it may not be a very difficult matter to avoid it in future.   It is worth while to contrast this with chemical analysis, wherein the content of samples is determinate with almost any degree of accuracy, but the material is destroyed in the process, and mechanical or physical defects can hardly ever be brought to light.

' What, then, is the information which can be obtained by the use of the microscope in engineering ?' The answer, so far as metals are concerned, may be given as follows : (1) Examination for defects, physical and mechanical, arising in manufacture. (2) Control of the heat treatment which the substance is to undergo in manufacture, and assurance of its suitability in the case of the finished product.   (3) Investigation of failures in use.   In the case of bodies other than metals the information sought is usually (1) Natural structure of the substance; (2) alteration of that structure in service.

Owing to their superior importance in most cases arising in engineering, we will take the metals first, though it is of interest to note that historically it was from the study of rocks that microscopy such as we are considering originally sprang.

Metals.

(1) **Defects arising in Manufacture.**—These may be taken to include blowholes, pipes, cavities, and all other forms of unsoundness due to faulty casting of ingots.   These troubles usually arise from the retention of gases, particularly hydrogen, which is trapped or occluded with vigour by many metals at temperatures near their melting-point ; and it is evident enough that such shortcomings, if incorporated into the finished article, will assuredly lead to failure sooner or later.   It is the business of the microscopist to investigate sections cut, both longitudinally and transversely, from ingots and castings, and to report upon the presence or absence of the above-mentioned blowholes, etc.

For this purpose it is often sufficient for a specimen of the metal
or alloy to be roughly ground down to a plane surface, and then
examined by means of a low-power objective (say, 1¼ inches),
the specimen being laid, if necessary, across the foot of the
microscope; the stage in such instruments should be removable,
in order that such work may be undertaken in the case of heavy
pieces. A vertical illuminator with transparent reflector between
objective-front and object is best for this work.

We have now to consider another type of defect likely to
arise in manufacture—*i.e.*, the inclusion of impurities. As an
example we will take the case of steel, though analogous instances
occur in most other alloys, and, of course, also in the case of
so-called 'pure' metals. Steel, as is well known, is a complex
alloy of iron, carbon, manganese, silicon, and phosphorus. The
presence of sulphur and phosphorus is due to metallurgical
processes into which it is unnecessary to enter, but if they occur
to an undue extent trouble is certain. Manganese in the form
of spiegel (Fig. 99) is added in steel-making to combine chemic-
ally with the sulphur, forming manganese sulphide, a compound
of less objectionable properties than ferrous sulphide, which
results if the amount of manganese is insufficient. The micro-
scope, skilfully used, will determine whether the above points
have been properly attended to by the steel-maker, for manganese
sulphide (MnS) appears under high magnification (say, 1,000
diameters) as dove-grey patches, whereas the ferrous sulphide is
blacker, but appears slightly yellow by reflected light. Neither
compound should occur in really first-class steel; but if it is
only the manganiferous impurity which is present, the results
are not likely to be so serious as if the sulphur-iron compound
were incorporated. Fortunately, the affinity of sulphur for
manganese is greater than that for iron, and therefore with
proper precautions it should be always possible to avoid the
formation of ferrous sulphide.

In cases such as this the microscopical technique needs to be
more refined than in the case of blowholes, etc. The specimen
should be comparatively small—about ½ inch square—and must
not only be ground, but polished, by being held against a
revolving wheel covered with fine cloth. The polishing process
is one that requires considerable skill, and if this part of the

procedure is unsatisfactorily done very misleading results may be obtained when the specimen comes to be examined under the microscope.

Numberless other cases occur where physical defects or impurities can be located with certainty, and the reader will notice that in neither of these two cases taken for illustration would chemical analysis have been sufficient : in the first instance, because such an investigation could obviously give no information as to the existence of cavities, etc.; and, in the second, for the reason that only the ultimate constitution of the steel would have been determined, and not the actual compounds which existed in the specimen.

Naturally, chemical impurities may present themselves in a great variety of ways ; but from the point of view of actual size such inclusions are usually small—the blue crystals of stannic oxide, fairly common in copper, for instance—and therefore a $\frac{1}{12}$-inch oil-immersion lens should be available, and a vertical illuminator mounted above the objective when such work is contemplated.

(2) **The Control of Heat Treatment** is one of the most important uses to which the microscope can be put in engineering practice. The properties of metals and alloys depend to an enormous extent upon the temperatures at which they have been cast, forged, annealed, or tempered ; and these temperatures are made manifest by the microstructure of specimens when properly prepared and examined.

At this stage it may help to make matters clearer if we consider very briefly the nature of these processes, for without some such knowledge it is impossible to appreciate their effects on the structure of the substance. Casting simply consists of the pouring of the molten metal into appropriate moulds. The temperature at which this is done is very important; if too high, the chemical activity of the substance may be largely increased, and it will take up impurities. If unduly low, pouring will be difficult, and the solid phase will have begun to separate before the mould is completely filled. Forging, or the working of metallic objects, must be performed between rigidly defined temperatures. If outside this range, it may be impossible to fashion the material, or else the internal crystalline structure of

the metal is injured by the succession of blows. Usually after forging the metal is in a state of strain (this may also happen from rapid cooling without additional work), and therefore this unstable state must be relaxed by annealing, or tempering, or both, such processes involving the reheating of the substance to appropriate temperatures.

We are now in a better position to appreciate the information which can be gained by the study of microstructures resulting from different heat treatments. We will begin by the consideration of steel, partly because of its importance in engineering, and partly owing to the fact that it responds in an unrivalled degree to heating and cooling effects. Now it is obvious that, if we wish to investigate microscopically the structure of steel near its melting-point (approximately 1,500° Centigrade), we must have some means of retaining at ordinary room temperature the conditions really obtaining at high temperatures. Technical applications and the pure interest of microscopy are together in requiring some such arrangement.

Therefore the process of "quenching" has been devised. This consists of taking the metal at any temperature desired, and plunging it into oil, water, or liquid air, the rate of cooling being least in oil and greatest in liquid air. Then (with important exceptions of too difficult a nature to be discussed here) the structure characteristic of the high temperature is retained in the cold : intermediate conditions of the body having been suppressed by the great rapidity with which the metal has been taken through the range of temperature. Incidentally this procedure illustrates a great natural truth, a characteristic of matter in general—namely, that a finite time is required for the transformations of nature to occur, and that if by any means we are able to expedite any process involving such changes so that the required time, however short, is denied to the substance, then the substance will continue to exist in the state characteristic of it when the process began, only in a condition of unstable, or metastable, equilibrium.

These different structures, characteristic of different temperatures and different rates of cooling, are those which we wish to investigate under the microscope. Let us take a few examples from the iron-carbon system of alloys. Imagine a ' mild-steel '—

i.e., one containing a very small percentage of carbon, which has cooled very slowly from the liquid state. All the intermediate conditions have had time to assert themselves, and the body— always supposing that the cooling has been slow enough—is in a state of stable equilibrium. When examined microscopically, it is seen to consist of different sized grains of pure iron (called by metallographists ' ferrite '), and, at the junctions, small dark inclusions (see Fig. 100). This is the constituent ' pearlite,' and is formed of alternate lamellæ of pure ferrite and tri-ferrous carbide (Fe_3C), the latter being known as ' cementite.' Mechanically, such an iron as this is weak; the large grains are the very reverse of a compact, close structure necessary to withstand the strains encountered in many branches of engineering. If we increase the carbon content, the areas of pearlite grow larger, until at 0·89 per cent. carbon the whole steel is formed of this constituent. Raising the carbon percentage still higher, the microscope shows coarse membranes of cementite interpenetrating the grains of pearlite; but in all cases the grain-size is large, as the result of slow cooling, and the material is ill-adapted for many purposes in engineering; though, of course, pure iron is very important in some branches of electrical work for magnets, where intensity of magnetism is more important than high retentiveness.

We may now consider the microstructure of a steel which has had work done upon it at an appropriate temperature and has been thoroughly annealed to remove strain. The large ferrite grains are conspicuous by their absence (Fig. 101), and, instead, we obtain a close-grained structure (needing a magnification of some 100 diameters or more to resolve it), generally the condition most favourable to strength and good physical qualities. In these cases, by suitable heat treatment, followed by microscopical analysis, we are able to produce and control the manufacture of steel having nearly any desired properties, in the way of wearing qualities, resistance to shock, resilience, toughness, or whatever may be the special characteristic desired. It is hardly necessary to point out how great is the power of the microscope, both in making sure that the structure is appropriate for any given purpose, and also, in the case of manufacture, in enabling metallurgists to try experiments with a view to producing some metal

or alloy having some desired property. This is of the greatest importance in the case of the so-called 'light-alloys' for motor-car and aeroplane work, in which branch of technology there is a great demand for alloys of aluminium combining strength with lightness. In these cases recourse to the microscope has been of the greatest assistance.

Amongst the problems often encountered under the general heading of heat-treatment is that of recognizing and studying the properties of intermetallic compounds. These bodies are peculiar, not to say remarkable. In the first place they very seldom conform to any accepted rule of chemical valency (though some reasons have been brought forward to account for this), and secondly, they are only stable within well-defined ranges of temperature, so that, in cooling, an alloy may consist, at different periods of its history, of quite different constituents; these breaking up, as the temperature falls, to give place to the next compound characteristic of the particular temperature range. Since heat is always liberated or absorbed in such changes, a study of the cooling curve together with microscopical examination of specimens quenched at the appropriate moment is very fascinating, for it is then possible to observe the existence of some compound just before an evolution or absorption of heat, and with the next specimen to see a structure entirely different, though these materials may only have been quenched at temperatures a few degrees apart. Since the physical properties of these intermetallic compounds are very different, quenching and microscopical analysis can obtain results of technical importance. Fig. 102 shows crystals of the intermetallic compound, iron-zinc.

3. **Investigation of Failures in Use.**—It is now necessary to enquire into the nature of the information which the microscope can afford us as to the effects of wear and tear made evident by microstructure. Firstly, it is well to point out that much of this work is of a controversial character, and therefore, in an article such as this, only opinions which have gained general acquiescence will be mentioned. There is a vast and increasing literature of this subject to which the reader is referred for more detailed treatment. Perhaps the most interesting case is that of 'fatigue.' It is a well-known fact that metals and alloys forming the moving parts of reciprocating engines, rotating

shafts and pulleys, etc., suddenly fracture without apparent reason, and when this occurs, a thorough microscopical investigation, together with chemical analysis, will often go a long way towards elucidating the cause of the accident. If steel is the material, the pearlite will generally be found to be markedly distorted, being dragged out or compressed, as the case may be, in the direction of motion. The author has himself investigated an instance—by no means uncommon—where a structure of parallel bands of pearlite has resulted from 'fatigue.' This is in marked contrast to the granular appearance, to which reference has already been made in the subsection dealing with heat-treatment (compare Figs. 103 and 104).

The general theory of distortion of metals under stress is very complicated, but it may be mentioned that the view most usually accepted is that the crystals of the substance are caused to slide bodily over one another, and a system of so-called 'slip-bands' results, when such a strained specimen is examined with a fairly high-power objective. In order that this slipping should occur, it is necessary to postulate the existence of an 'amorphous cement,' which is supposed to fill the spaces between adjacent crystal grains. The microscope has never yet been able to demonstrate the presence of such a material, but the agreement between actual behaviour of a strained metal and the predictions of theory based on the assumption that an amorphous layer is included at the junctions, is so good that it may only be a question of time before its existence is placed beyond doubt. Again, in the case of metals exposed to the influence of chemically active gases (i.e., in flues, blast-furnace pipes, and in case-hardening), the microscope has demonstrated a gradual absorption of carbon from the edge of the specimen inwards.

The reader will no doubt have come to the conclusion by this time that the usefulness and importance of the microscope as an instrument in the metallurgical side of engineering are very great, but before proceeding to a discussion of the actual technique and manipulation involved, some reference is required concerning the part played by the microscope in problems bearing on the second class of material mentioned early in this article—namely, refractories or non-metallic substances.

It is difficult to assign a reason why progress in this direction

has been slower than in the case of metals, more especially since—for many purposes—the type of microscope required is simpler. However, a mass of information has been accumulated, of which the barest outline can be given here.

Non-metallic Substances.

Ganister, the most important, is a hard silicious rock used in lining furnaces, Bessemer converters, and for various other purposes. The best quality contains the greatest percentage of silica (SiO_2), the usual range being anything between 80 and 98 per cent. In the case of three specimens in the examination of which the author was concerned it was quite easy, with low magnification (about 10 diameters) and the usual transparent illuminating arrangement, to note the differences of silica content; and, from that, to advise the use of one material more than another, for any specific purpose. Fortunately ganister is transparent when crushed, and resembles quartzite in microstructure when the percentage of silica is very high.

Dolomite.—A mineral rich in magnesium carbonate, and used as a lining in basic steel manufacture, is also amenable to microscopical inspection in much the same way as ganister, and the information obtained with respect to grain-size, compactness, and other desirable properties is often very useful.

Slags.—In recent years, too, the study of the microstructure of slags has been actively taken up. A prodigious amount of slag is necessarily produced in steel-making, and as there is no very great demand for this substance for ballast, a large portion of the output is taken out to sea and 'drowned.' It is therefore becoming of increasing interest to enquire into its constitution with a view to finding more uses for this by-product. The manufacture of slag-wool is an industry of increasing value, and may well provide more problems for the microscopist. Slags are examined either by transmitted light, as in the case of ordinary transparent preparations, or else with the vertical illuminator. The structures observed are of the very greatest beauty, and are not unlike the radiating and spherulitic crystals familiar to microscopists who study chemical salts with the aid of polarized light. In some cases very rare minerals have been

found in these slags, and it is not uncommon for this artificial silicate to resemble closely igneous rocks formed at considerable depths from a molten magma in the earth's crust.

Cements.—These also have come in for their share of attention, and the property of 'setting,' so long a mystery, has been very largely accounted for by microscopical analysis. There is now little doubt that different hydrates, corresponding to different degrees of supersaturation, are among the causes that contribute to the characteristic of 'setting.' By subjecting cements to microscopical investigation a great deal has been learnt about these hydrates, and the conditions under which they are formed and can exist in stable equilibrium (see Fig. D, Frontispiece).

The Metallurgical Microscope.

It is now proposed to devote some attention to the methods of microscopy which experience has suggested when dealing with metals. In the first place, let us consider—

The Stand.—The primary essential is that it should be rigid —not that this is a novel requirement—but it is placed before others because many a good instrument is wellnigh useless, owing to the fact that when heavy pieces of metal are being examined the balance is disturbed, and high-power work especially becomes next to impossible. It is important to remember that in a great number of cases microscopical investigations are carried on in works laboratories, where the conditions are not ideal, owing to the working of machinery in the immediate neighbourhood and consequent liability to vibration.

The Stage is the next consideration. It should be large, have mechanical movements in two directions, and also a raising and lowering rackwork, so that coarse focussing can be done with the stage, instead of by moving the body-tube (see Fig. 105). This arrangement prevents the disturbance of the illuminating system with opaque objects.

As previously suggested, it is an additional convenience if the stage is removable, or alternatively provided with a large central opening, so that bulky specimens can be observed by laying them across the foot. It is quite unnecessary for the stage to rotate, though some of the more advanced instruments include this feature. The stage suggests specimens and methods of mounting.
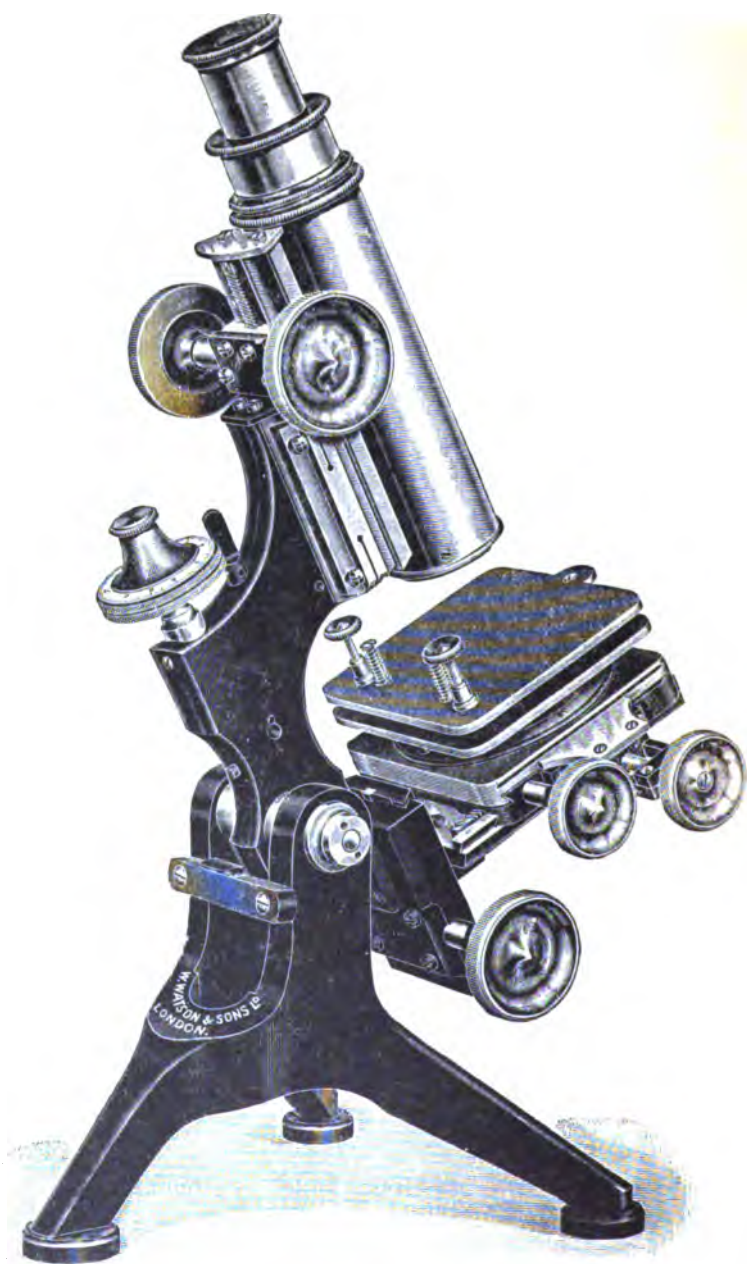
FIG. 105.—A TYPICAL METALLURGICAL MICROSCOPE.

Mounting Metallurgical Sections.—Since the majority of these are usually plane only on one surface and rough on the others, one quite efficient method of levelling is to take an ordinary 3-inch by 1-inch glass slip, put on it a piece of plasticine, place the specimen face downwards on a flat piece of glass at the bottom of a ring, and press the plasticine down on to the rough face of the object, the glass slip resting on the edges of the ring. Other more complicated arrangements are made for the purpose of levelling specimens, but, taken on the whole, the simple expedient just described is quite efficient.

The **Vertical Illuminator** is referred to in another part of this book, but by way of supplementing the information given there, mention might be made of the condenser pattern (Fig. 106).

Two good points in favour of this arrangement are that, con-

FIG. 106.

taining as it does its own lighting system, the whole apparatus can be attached to the body-tube of an ordinary microscope (moving with it, and therefore dispensing with the need for a movable stage), and also that mounting of objectives in short barrels is rendered less essential than with other patterns of vertical illuminator.

Objectives.—First quality achromatic lenses are quite sufficient for most purposes, though for the highest-power photomicrography apochromats are no doubt superior. An objection to the latter is the curvature of field, particularly annoying when specimens have to be examined over large areas. Restricting our attention to achromatic objectives, the most useful powers will generally be found to be 2 inch, 1 inch, $\frac{2}{3}$ inch, $\frac{1}{6}$ inch, $\frac{1}{12}$ inch (oil-immersion). Owing to the fine structures sometimes occurring, lenses with high N.A. are preferable throughout.

One more point is very important. In designing objectives for opaque objects, it is a great advantage if the back lens be

made as convex as the rest of the design will allow, for this throws reflected light towards the walls of the tube, and thus tends to reduce flare.   A black velvet lining to the draw-tube may also assist.

Eyepieces.—These should be as low in magnifying power as possible.   If greater magnification is needed, it is best attained by a higher-power objective.   The author seldom uses an ocular of greater strength than × 8.   Compensating or special types of eyepieces are generally unnecessary.

If an eyepiece micrometer is to be used for measuring purposes, it must be very deeply ruled or etched, for the contrast against a metal specimen is feeble, and reading will be found difficult.

Illuminant.—Information as to suitable light-sources may be welcome.   The author uses a 'Half-Watt' lamp (electric) and finds it admirable.   The 'Pointolite' tungsten bulb is generally considered the best of all.   Even a Welsbach gas mantle is very efficient if electric light is unobtainable, but in any case the illumination must be intense, and a bull's-eye condenser (focussed through a vertical illuminator upon the back lens of the objective) is essential, unless the condenser illuminator is used.

Etching.—In order to develop the microstructure, polished specimens are usually treated with an etching reagent, and dried by means of an air blast.   Various preparations, generally solutions of acids or alkalies, are used.   The mechanism of the process is complicated.   It most probably depends upon the setting up of 'contact electromotive forces' at the boundaries of adjoining grains.

In conclusion, it is hoped that this brief outline of the part played by the microscope in engineering will be sufficient to show the possibilities and problems that await workers in this field of research.

THE MICROSCOPE IN AGRICULTURE

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AGRICULTURE—the oldest and perhaps the most important industry—has in the past received far less attention from the trained scientist than any of the activities designed to benefit man. But now that the various agricultural problems, and the empirical methods long practised by the farmer, are being more fully investigated, it is becoming evident that some of the most complicated and interesting scientific questions are waiting to be solved.

In the early days of research, attention was largely directed towards the chemical aspects of the soil, and Liebig taught that the nitrogenous organic matter was decomposed by a chemical process involving the production of ammonia. During the late sixties and early seventies, however, bacteriology made great advances, and it was demonstrated that all decomposition and purification was largely due to the activities of bacteria. From this the deduction was made that the decomposition in the soil was probably brought about by the same agents. Schloesing and Muntz, in 1877, experimentally showed that in sewage beds the conversion of ammonia-into nitrate was not a chemical but a biological process ; while Warington, working on Rothamsted soils, proved that it involved two stages associated with two distinct organisms; but not until 1890 did Winogradsky, by a series of brilliant experiments, isolate these two micro-organisms in artificial culture.

This date may be termed the beginning of the biological epoch in agricultural research, and since then this aspect of soil fertility has assumed more and more importance.

It is now well established that soil fertility is dependent on the activities of the bacteria; as already stated, the production

of nitrate from ammonia is dependent upon them, as also the decomposition of carbohydrates. Further, recent research has demonstrated that such substance as naphthalein and phenol, when added to the soil, are broken up by particular species of bacteria.

Unfortunately the short space at my disposal renders it impossible to deal even briefly with agriculture in general, or with the various classes of micro-organisms found in the soil. Since, however, bacteria are dealt with in other sections of this book, and since to-day practically everyone knows something about them, I propose to confine my attention to the less known organisms.

We know that every gramme of soil must be regarded as the abode of a vast population. Besides the earthworms and numerous species of insects, there are large numbers of protozoa, algæ, and fungi. For a correct solution of many soil problems, it is essential that we know something of the life histories of these micro-organisms as well as of their inter-relationships. Such information can only be obtained by the use of the microscope, coupled with a knowledge of how best to use the various mechanical and optical combinations which have been devised. For these reasons, therefore, it is felt that no apology is needed for the inclusion of a chapter on agriculture in a book of this nature.

Protozoa.—The study of soil protozoa is a growth of comparatively recent origin, and arose out of the investigations as to the cause of increased soil fertility after its treatment by various external agencies. As early as 1888, Frank attempted to explain the increased crop production of soils treated with steam on the assumption that the chemical changes so induced were the operative factors. Numerous other observers, such as Richter, König, Stone, Koch, Peterson and Johnson, adopted this view, but no uniformity of explanation emerged from the work, and serious objections to such a view can be adduced. The treatment of soils by antiseptics, instead of steam, also increases the yield, but contradictory results and opposed explanations were the rule in the early days of investigation. It may, however, be definitely stated that, by heating, the physical, chemical, physiological, and bacteriological properties

of the soil are more or less altered; coupled with which there is an increase in the soluble matter—both organic as well as inorganic—available for plant growth, and, by the use of antiseptics, important changes in the flora, especially respecting nitrification, are produced.   Various theories have been put forward to explain these results, though only one need be considered here—viz., that of Russell and Hutchinson, which was initiated in 1905 by the publication of a paper by Russell, followed by a second one by Russell and Darbyshire in 1907. Here it was shown that the absorption of oxygen by the soil is mainly brought about by the action of micro-organisms, and is greatly diminished if the soil has been previously heated to 120° C.   After heating, however, to 95° C., the rate of oxidation, instead of being reduced, is considerably increased, in some cases being 50 per cent. greater.

Russell and Hutchinson then began an extended series of experiments on the bacterial numbers of such soils.   It was found that treatment, either by heat or antiseptics, effected a 'partial sterilizing' action in that some groups of bacteria were destroyed—e.g., the nitrification organisms; but the decay bacteria and those producing ammonia were still present. Moreover, a count of the number present in the soil showed invariably, that though a depression was at first produced, rapid reproduction soon took place, until eventually the numbers considerably exceeded those present in untreated soil (see Table I.).

TABLE I.—BACTERIA IN MILLIONS PER GRAMME OF DRY SOIL.

	At Start.	After 16 Days.	After 30 Days.	After 74 Days.	After 200 Days.
Soil 1 :					
Untreated	27	10	10	45	—
Treated with carbon di-sulphide	2	17	58	121	—
Soil 2 :					
Untreated	18	9	4	9	12
Heated to 65° C. ...	18	21	87	45	60
Soil 3 :					
Untreated	11	16	9	18	6
Treated with toluene ...	2	48	41	48	18

Various experiments demonstrated that in normal soil there is resident a factor detrimental to bacterial development, which factor is eliminated in partially sterilized soil by the treatment employed. Further, this factor possessed the following properties:

(a) It is active and not the lack of something.

(b) It is not bacterial.

(c) It is extinguished by heat or poisons.

(d) It can be reintroduced into soils from which it has been removed by the addition of a little untreated soil.

(e) It develops more slowly than bacteria, and for some time may show little or no effect; then it causes a marked reduction in the numbers of bacteria, and its final effect is out of all proportion to the amount introduced.

(f) It is favoured by conditions favourable to trophic life in the soil, and finally becomes so active that the bacteria become unduly depressed; this is one of the conditions obtaining in greenhouse 'sick' soils. It is difficult to see what agent other than a living one could satisfy these conditions. Search was therefore made for soil organisms larger than bacteria, and the protozoa were provisionally regarded as fulfilling the necessary requirements.

Thus the impetus given to the study of soil protozoa must be ascribed to the work of Russell and Hutchinson.

The general biologist has long been familiar with these lowest forms of animal life; they have long held an important place in medicine and veterinary science, but they have not as yet been generally regarded as soil organism worthy of serious attention. At present it must be recognized that the significance and effect of the protozoa in the soil is far from understood. Many and varied are the criticisms levelled against the theory of Russell and Hutchinson, but in no single instance has the adverse evidence adduced been sufficiently strong to justify abandonment of their view.

What is required is a solid foundation of pure soil protozoology, quite irrespective of any applied bearing it may ultimately possess, for only by such means will any advance be made. This is undoubtedly a truism, since so-called 'applied' science, if it is of any worth, is simply the application of 'pure' science to particular kinds of problems.

Practically all the species of protozoa resident in the soil possess in their life history two distinct phases—the cystic and the active or trophic. In the first the organism assumes a rounded shape, secretes a thick wall, and remains in this quiescent condition for varying periods; but in the second or active stage the animal moves, feeds, and reproduces. Now in the early days of the work it was asserted that bacterial numbers could not be affected by protozoa, because the latter existed in the soil only in the cystic condition. Martin and Lewin, however, by ingenious experimental methods, demonstrated that there are in Rothamsted soils both active flagellates and amœbæ. Critics of Russell and Hutchinson's hypothesis maintained that the soil protozoa must be very few in number, since it was impossible, on examining soil under the microscope, to find any trace of them. The present writer has shown, however, that this is due to a surface energy relationship existing between the soil particles and the protozoa, so that the two are always in intimate contact. In other words, the organisms have the film of water surrounding the particles as their environment, which is probably different in character from that of the free spaces of the soil—a fact that must always be considered when discussing physiological processes.

Although the qualitative investigations of Martin and Lewin were at the time valuable, in order to make further advance it was necessary to devise a means whereby the numbers of protozoa in a given soil sample could be ascertained. Such a quantitative method was, however, difficult to provide on account of the relationship between protozoa and soil particles. A direct count of the numbers obviously could not be made. Indirect means have, however, been found depending essentially on dilution methods. In one of these, devised by Cunningham and modified by L. M. Crump, 10 grms. of soil are added to 125 c.c. of sterile tap water and shaken for three minutes. This gives a 1 in 12·5 dilution. From it further dilutions are made until a sufficiently high one is attained. Petri dishes, containing nutrient agar, are inoculated with 1 c.c. of each of the dilutions and incubated. At intervals of 7, 14, 21, and 28 days the plates are examined, and the presence or absence of protozoa on each recorded. In this way it is possible to calculate approximately the

numbers of protozoa present in each gramme of the soil investigated.

By means of some such quantitative method the numbers of bacteria and protozoa have been counted, and numerous papers published giving the results obtained. They are, however, inconclusive: thus, on the one hand, Goodey and several American observers concluded that protozoa were probably not agents in depressing bacterial numbers in normal soils, while Miss Crump and Cunningham obtained evidence pointing to the reverse conclusion. Such divergence of opinion was probably mainly due to two causes: firstly, the periods elapsing between the examinations of the soil samples were too great; and secondly, all the counting methods were unsatisfactory in that they were unable to differentiate between the cystic and active phases of the protozoon life cycle. This is a particularly serious source of error since it is possible in a given sample to have a large number of bacteria, and a large number of protozoa, 90 per cent. of which are as cysts. A count on such a sample would give a result apparently entirely opposed to the theory of action and interaction between protozoa and bacteria, though in reality, as only 10 per cent. of the protozoa are active, and therefore capable of affecting bacterial numbers, the theory would be upheld.

The difficulty has, however, been recently overcome by a further modification of the dilution method. Briefly it consists in dividing the samples into two 10-gr. portions. One of these is counted in the usual way, thus giving the *total* number of protozoa (active + cystic) present. The second portion is treated overnight with 2 per cent. hydrochloric acid (which experiment has shown kills all active forms, leaving the cystic ones unharmed), and counted as before. This gives the number of cysts which subtracted from the total gives the active number. * By employing this method in an extensive experiment striking results have been obtained to be described later. First of all, however, a short account may be given of the types of protozoa found most commonly.

* The details of this method and proof of its accuracy will be found in the following papers :
  1. Cutler, D. W. (1920), *Journ. Agric. Sci.*, x. 186-148.
  2. Cutler, D. W., and Crump, L. M. (1920), *Ann. App. Biol.*, vii. 11-24.

Martin and Lewin, and Goodey first demonstrated that in Rothamsted soils there exist representatives of the three large groups of Protozoa—viz., the Rhizopoda, the Ciliata, and the Mastigophora. Since then it has been shown that these organisms are very widely distributed, occurring in soils from all parts of the world, and that this obtains, not only for the group, but also for certain species.

Of the Rhizopoda two species of amœbæ appear to be dominant; one of them, described by Martin and Lewin as *Vahlkampfia soli*, is undoubtedly *Dimastigamœba gruberi*, originally described by Schardinger in 1899.

The second dominant soil amœba, not yet described, is much smaller and occurs less frequently. Other species, though less in number, are *Amœba glebæ* (Dobell), *Amœba lawesiana* (Goodey), *Amœba agricola* (Goodey), *Amœba cucumis* (Martin and Lewin), *Biomyxa sp.*, and *Nuclearia denticularia*.

Among the Mastigophora the species most frequently met with are: *Oicomonas termo* (Ehren), *Cercomonas longicauda*, *Heteromita sp.* and *Monas sp.*; and, sporadically, *Tetramitus spiralis*, *Tetramitus rostratus*, *Spiromonas angusta*, *Copromonas sp.*, *Proleptomonas fœcicola*.

The Ciliata are represented by two species of Colpoda: *C. cucullus* and *C. steinii*, *Gastrostylis affinis*, *Pleurotricha grandis*, which are invariably found, and by less commonly encountered forms such as *Vorticella microstoma*, *Anophrys sp.*, and *Euplotes carinata*.

Such then are a few of the soil protozoa; the list is by no means complete, but indicates the complexity of the fauna.

Let us now consider some of the results obtained in the recent experiment referred to above. It was extensive, in that soil samples were taken daily for 365 days and the numbers of bacteria counted. Further, the species of protozoa were differentiated and counts also made of the numbers of active and cystic forms of the dominant ones. In this way a great deal of data was accumulated, consisting of about 16,000 sets of figures. With such a collection it is neither possible nor suitable to deal in an article of this nature except in the briefest manner. I shall, therefore, confine myself to giving some of the salient facts.

Firstly, it was found that the bacterial numbers changed

considerably from day to day. Previously it had always been assumed that their numbers remained relatively constant, experiencing only seasonal fluctuations. Thus it was known that in the autumn and spring there was an increase in the bacterial population, but that more frequent variations occurred was not recognized. The recent work demonstrates, however, that the variations from day to day are in many cases as much as or even more than 100 per cent. (Table II.).

TABLE II.

Sample.	Bacteria in Millions per Gramme.	Active Amœbæ.	Sample.	Bacteria in Millions per Gramme.	Active Amœbæ.
223	18·18	386,800	280	15·8	288,000
224	21·2	127,000	281	19·75	157,000
225	12·6	227,000	282	29·8	Under 50
226	13·5	221,900	283	40·5	Ditto
227	8·8	238,000	284	35·2	25,000
228	15·7	59,800	285	29·3	42,000
229	42·0	2,826	286	54·5	2,686

This has an important bearing on many previous investigations where an interval of days or even weeks elapsed between successive counts; because of this some of the conclusions deduced from such experiments will have to be revised.

When in relation to the bacterial numbers those of the active amœbæ are considered, it is evident that a close connection exists between the two. Throughout the whole of the 365 consecutive counts, with negligible exceptions, when the bacterial numbers were low those of the active amœbæ were high, and *vice versa.* This is illustrated in Table II. and Fig. 107.

It may be said, therefore, that the general principle holds that there is in normal soil an inverse relationship between the numbers of bacteria and of the active amœbæ. As I view it, in such soil there is always an endeavour to obtain equilibrium between the two sets of organisms; but, possibly because of varying external conditions, or more probably because of the sequences composing the life cycle of a protozoon, the poise of the balance is continually being upset. Thus, for some reason, not yet entirely understood, the amœbic population increases, whereupon the bacterial numbers are depressed, while at a new
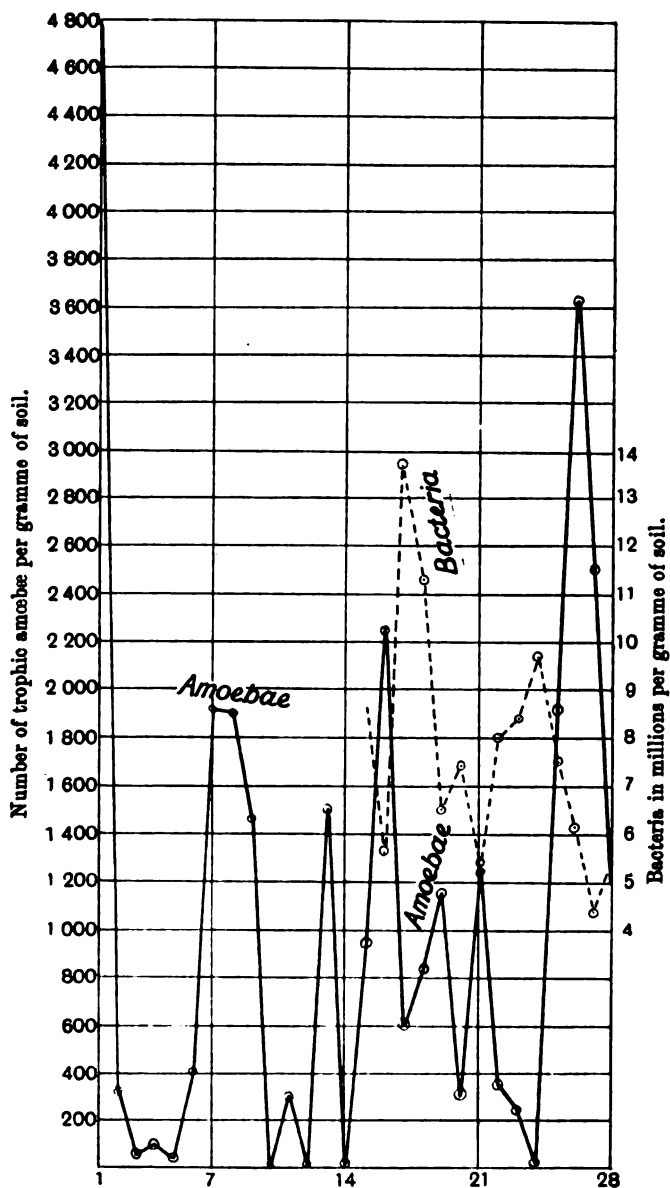
FIG. 107.—NUMBERS OF BACTERIA AND TROPHIC AMŒBÆ IN BROADBALK, PLOT 2, FROM FEBRUARY 9 TO MARCH 8, 1920.

Daily countings. (Reprinted from the *Ann. App. Biol.*, vol. vii.)

phase of the life cycle, or because of an inimical environment, the amœbic numbers are lowered to be followed by bacterial increase.

It might, perhaps, be said that the amœbæ are the cause of the lowering of the bacteria. This would, doubtless, to a large extent be correct, but the word 'cause' is dangerous since it implies a straight and unalterable issue between bacteria and protozoa. There is little warrant for such a view; nor is so simple a conception likely to be a true one; rather should it be said that the relationship is one factor in a long chain of events leading to soil fertility. As Professor Arthur Thomson said in his Gifford lectures: 'No creature lives or dies to itself, there is no insulation. Long nutritive chains often bind a series of organisms together in the very fundamental relation that one kind eats the other.' In soil such 'nutritive chains' obtain just as markedly as in other haunts of life.

Turning now to the flagellates, an entirely different state of affairs is revealed. It is impossible to establish a direct correlation between them and the bacteria, though undoubtedly a small depression is brought about, since some of the species feed on bacteria; the effect is, however, so slight that it is completely masked. All the species investigated, however, exhibit the daily fluctuations in number, but with one exception the variations appear to be quite irregular. *Oicomonas termo* (Ehren) is a marked exception. As will be seen from Fig. 108, there is a two-day periodicity in respect to the active numbers of this animal, high numbers on one day being succeeded by low ones on the following day, and then by high ones again on the third day. This rhythm has been maintained throughout the year's count, and also obtains in artificial cultures kept under constant laboratory conditions. It is not possible to correlate this rhythm with any of the obvious external conditions, and probably we are dealing with a factor inherent in the organisms and bound up with the life cycle.

Oicomonas termo has two methods of reproduction; asexual, in which by a simple division two daughter animals are produced, each of which may grow into an adult *Oicomonas;* and sexual. In this last method two of the organisms fuse into a common mass around which a thick-walled cyst is formed. After an

interval, out of this cyst a single individual is born.   As already
stated, the rhythm may possibly be explained in terms of such a
life cycle, but this cannot be definitely stated owing to our lack
of knowledge of it in terms of *time*.

The series of events leading to cyst formation we know, but
not the period of time necessary for it.   This can only be
discovered by work on pure culture, a beginning of which has
been made.   I would, however, point out that periodicity is a
widespread phenomenon in biology, and in particular as regards
reproduction.
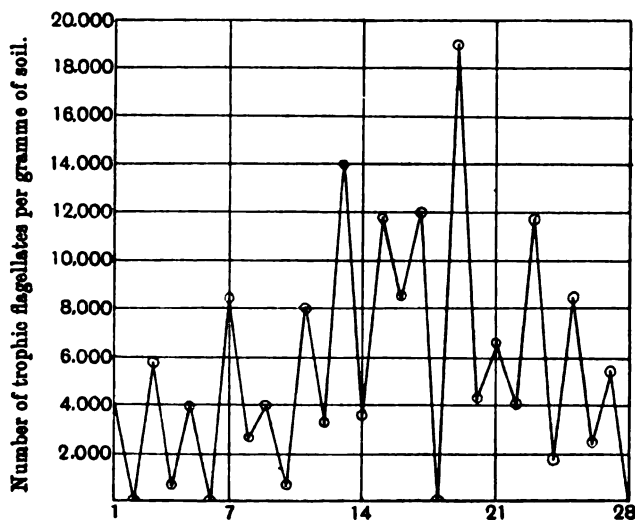
FIG. 108.—ACTIVE NUMBER OF OICOMONAS TERMO (EHREN).
Daily countings. (Reprinted from *Ann. App. Biol.*, vol. vii.)

Before leaving the discussion of soil protozoa there are two
other interesting points to be mentioned.   As I have already
stated, the periodicity of *Oicomonas* and the obvious external
conditions do not seem to be correlated; nor does it appear
probable that the daily variations in the numbers of the other
species of protozoa can be explained by reference to environmental
changes.

At present these fluctuations can be accounted for only on the
supposition that the operative factors are inherent in the
organisms themselves.   A great deal of research is necessary to
settle this point, but it is of fundamental importance both to

biology and for the elucidation of some of the problems connected with agriculture.

Superimposed on the daily variations there are seasonal ones. Soil bacteria have long been known to exhibit autumn and spring rises, but only recently has it been shown that the protozoa go through the same changes. As regards the two dominant species of amœbæ, the total numbers rise at approximately the same time as do the bacteria; this might be explained as due to an increased food supply; but the increase in the total numbers of flagellates does not synchronize with that of the bacteria, each species having its peak at a different time. At present this phenomenon is unexplained.

I must now leave the question of the soil protozoa and, in the short space at my disposal, deal with the algæ and fungi.

Algæ.—The algæ of the soil have until the last few years been practically entirely neglected. This is possibly due to the assumption that they could in no way be a factor in soil economy; but recent investigation has shown that in all probability they are, from the agricultural point of view, well worthy of research. They, like the protozoa and bacteria, are found to a depth of about 12 inches, though not uniformly distributed, occurring in greatest numbers in the intermediate depths. They have been obtained from all the soils in which they have been sought, but especial attention has been given to the soils of the German African colonies and Schleswig-Holstein by Esmarch, to those of Colorado by Robbins, to the Danish soils by Peterson, and to the Rothamsted ones by B. M. Bristol.

The flora, largely composed of the blue-green and green algæ and diatoms, is especially extensive in cultivated soils; and Esmarch concludes that their not being confined to the surface layer is due to cultivation, in that these operations tend to bring fresh soil constantly to the top with the consequent burying of the surface algæ. As other effective factors the burrowing of worms and the percolation of water are cited.

The fact that plants containing chlorophyll are capable of continuing vegetative growth in the darkness of the soil is surprising, but it has been demonstrated that this can occur, provided that an adequate amount of nitrogenous material is available. Also, Esmarch found that the blue-greens retained

their colour for some weeks, though eventually they became yellowish. There is no doubt but that the algal flora is not confined to the top layers of the soil, but that some species at least are capable of a vegetative existence in the lower layers. It is probable, however, that, under these conditions, they function a little differently; those on the surface will assimilate CO_2 from the atmosphere and accumulate carbohydrate material and energy, whereas those in the dark will consume such material.

Many species of algæ have a phase in their life-history called the 'resting condition.' This, in many ways, corresponds to the cystic stage of the protozoa; for when in this resting condition they have the power of retaining their vitality for long periods under unfavourable conditions. Thus Miss Bristol found that two species, *Nostoc muscorum* and *Nodularia Harveyana*, resumed growth after seventy years' desiccation in Rothamsted stored soil, while many species were viable after forty-seven to fifty-nine years' storage.

Finally, a consideration of the probable functions of soil algæ may be of interest. Frank, Schloesing, and Laurent asserted that they were able to fix atmospheric nitrogen as do species of bacteria. Kossowitsch showed conclusively, however, that this was not the case, but that the presence of algæ in certain soils was highly advantageous to nitrogen fixation. He concluded that this enhanced fixation in the presence of algæ was due to a symbiosis between them and the bacteria, the algæ supplying carbohydrate material to the bacteria from the mucous sheaths surrounding certain species, and the bacteria providing their partners with nitrogen, without which they are unable to develop. Such a view is substantiated by other experiments. Thus Boulhac and Gustiani showed that in sand, devoid of organic matter and nitrogen compounds, algæ and soil bacteria in combination were able to develop normally, and so to enrich the sand that it was capable of carrying a crop of higher plants.

Also, it must be remembered that by their death the algæ enrich soil in that they present to the putrefactive bacteria large quantities of organic matter for decomposition. This may have a particular significance in certain parts of the world—for instance, it is well known that the water of the Nile renders the land very fertile, but is itself deficient in organic matter and

nitrogen. On the other hand, vast numbers of algæ are present in it. It may be, therefore, that the fertilizing power is largely due to these forms of plant life.

Further, it must be remembered that by photosynthesis—the taking in of carbon dioxide and the giving up of oxygen—and respiration the surface algæ have an influence on the soil gases. Thus Harrison and Aiyer, working on swamp rice soils, concluded that the film of algæ covering the surface induced aeration of the roots of the crop by the oxygen evolved, which was then dissolved in the irrigation water, being carried to the roots. Such, then, are a few of the results achieved by the study of soil algæ. The investigations are in their infancy as yet, but there is little doubt that through further research the importance of algæ will become more and more evident.

Fungi.—As is well known, this group is of enormous importance in relation to diseases of plants, but with this aspect of agriculture I do not propose to deal. Of the fungi normally living in the soil we know little, though it has long been recognized that there is a large population, numbering more than 200 species. They have, however, been scarcely investigated as regards their function in the soil. This is partly due to the difficulties attendant upon such a study, for suitable methods have not yet been devised. For instance, it is not possible at present to make an accurate count of their numbers in a soil sample; also, as artificial media is employed for their cultivation, there is no means of ensuring that all the species present will grow. This, of course, applies to research on the protozoa and algæ, but with not the same force as in the case of fungi.

In spite, however, of the various difficulties, a good deal of work is now being done on these organisms. The genera most commonly found in temperate zones are Mucor, Penicillium, Fusarium, Aspergillus, Cladosporium, and Trichoderma, though sporadically many others occur. As many of these fungi have the power of withdrawing ammonia from the ammonium salts contained in manure, thus setting free acid, much of the acidity produced in land long dressed with ammonium salts may be ascribed to them. Like all living organisms, fungi require energy, which they obtain from carbohydrates. Unlike the bacteria, however, many species can use cellulose as their

source of supply, and are thus responsible for a certain amount of the decomposition of plant residues.

It was once held that fungi were capable of fixing atmospheric nitrogen, but this has been shown not to be the case, with the possible exception of one species, *Phoma betæ*. The source of nitrogen supply is amino-acids, so long as the amount of carbohydrate material available is sufficient; when this is not so, Waksman claims that the fungi can use soil protein compounds.

Finally, mention may be made of the mycorrhiza, fungal filaments found in association with the roots of higher plants. According to Frank, the fungus attacks the humus and mineral sources of the soil, passing them on as food to the plant. The extreme case of dependence is shown by some species of orchids which possess no leaves, relying practically entirely on the fungi for much of its food. Such a condition is not normal, however; usually the host plant is able to obtain its nutriment unaided when growing in favourable conditions, only assuming the symbiotic habit when the soil is poor, as, for example, in heaths and moors. Stahl, who has considerably extended our knowledge of these interesting fungi, shows that this co-operative method of living is very widespread, and is especially characteristic of plants growing in dry soils—those poor in mineral salts or rich in humus. Though not of direct agricultural importance, yet this question of mycotrophy has an important practical application. There are many plants, such as the orchids, lilies, etc., which must be grown in soil rich in humus; when, however, this is supplied, development of such seedlings does not always take place, probably because the appropriate and necessary fungus is lacking. If, however, a little of the soil in which the parent plants are growing is mixed with the soil in which the seeds are sown, germination occurs, and mycorrhiza are found at a very early stage of growth.

As a result of this brief survey of some of the micro-organisms it must be realized that the soil is by no means an inert mass of dead material, but rather a laboratory where very complicated chemical reactions are continually going on through the agency of the living population. The soil, even when undisturbed, is never in a state of equilibrium; and when one also considers the complications brought about by the different agricultural

operations, it becomes evident how difficult a business it is to obtain any clear conceptions of the changes going on, or to formulate definite hypotheses as to the chain of events leading to soil fertility, and ultimately to the crop of wheat covering a field.

Many aspects of agriculture I have had perforce to omit, but if I have demonstrated the importance of biology, and especially the importance of the microscope, when in the hands of those trained in its use, the purpose of this chapter will have been served.

ADDENDUM

For those who wish to extend their reading in the branches of agriculture dealt with above the following references to literature are given. These papers also contain a fairly full bibliography:

General Agriculture.

HALL, A. D. (1920). 'The Soil,' third edition. John Murray, London.
RUSSELL, E. J. (1921). 'Soil Conditions and Plant Growth.' First volume of the Rothamsted Monographs on Agricultural Science. Longmans, Green and Co., London.

Protozoa.

CUTLER, D. W. (1919). 'Observations on Soil Protozoa,' *Journ. Agric. Sci.*, ix. p. 430.
CUTLER, D. W. (1920). 'Method for Estimating the Number of Active Protozoa in the Soil.' *Journ. Agric. Sci.*, x. 135.
CUTLER, D. W., and CRUMP, L. M. (1920). 'Daily Periodicity in the Number of Active Soil Flagellates, etc.' *Ann. App. Biol.*, vii. 11.
CRUMP, L. M. (1920) 'Numbers of Protozoa in certain Rothamsted Soils.' *Journ. Agric. Sci.*, x. 182.
GOODEY, T. (1916). 'Further Observations on Protozoa in Relation to Soil Bacteria.' *Proc. Roy. Soc. B.*, 89, p. 297.
GOODEY, T. (1916). 'Observations on the Cytology of Flagellates and Amœbæ obtained from Old Stored Soils.' *Proc. Zool. Soc.*, p. 809.
RUSSELL, E. J. (1915). 'Soil Protozoa and Soil Bacteria.' *Proc. Roy. Soc. B.*, 89, p. 76.

Algæ and Fungi.

See accounts given in the books by Hall and Russell respectively, and :
BRISTOL, B. M. (1919). 'On the Retention of Vitality by Algæ from Old Stored Soils.' *New Phytologist*, xviii. 29.
BRISTOL, B. M. (1920). 'On the Algæ of Some Desiccated English Soils: An Important Factor in Soil Biology.' *Ann. Bot.*, xxxiv. 35.
BRIERLEY, W. B. (1919). 'Some Concepts in Mycology.' *Trans. Brit. Mycological Soc.*, vi. 204.
RAYNER (1916). 'Recent Development in the Study of Endotrophic Mycorrhiza.' *New Phytologist*, xv. 161.
STAHL (1900). *Jahr. f. wiss. Botanik.*, xxxiv. 643.

It is essential that anyone desirous of doing research on the micro-organisms of the soil should have a knowledge of modern bacteriological technique and of general physiological methods.

As regards apparatus, it is necessary to have a microscope stand made by a well-known firm, and fitted with a sub-stage condenser capable of being centred.   A mechanical stage will be found very useful. The objectives mostly employed are $\frac{1}{12}$-inch oil immersion; $\frac{1}{6}$ inch and $\frac{2}{3}$ inch, together with a set of eyepieces ranging from 4 to 18 magnification.   It is, perhaps, hardly necessary to emphasize the point that to get the best out of the microscope, a general knowledge of its construction and the optical principles involved is essential.

PART III

THE MICROSCOPE AND THE NATURALIST

INTRODUCTION

By WILFRED MARK WEBB, F.L.S.,

Honorary General Secretary of the Selborne Society.

YEAR by year the interest which is taken in the world around us, in the unspoiled works of Nature, continues to increase. It is now also generally recognized that to train the powers of observation is one of the most important necessities in general education, and that it is far better for everyone to teach themselves naturally through the interest aroused by the subjects considered, than to learn nothing but second-hand facts from others.

To the majority of people, whether they are children or not, living things prove most attractive. The general appreciation of them begins most easily and properly out of doors, and may continue as a lifelong pursuit. The detailed investigation of some part of natural history forms an interesting hobby as well as a healthy form of exercise, recreation, and relaxation, particularly for those who are not forced to spend all their spare time upon games, or to whom the more violent forms of athletics and the usual kinds of sport do not appeal.

Whatever line of study is taken up, it will be very soon found that if any real progress is to be made—if anything new is to be found out with regard to the structure of the various creatures apart from the obvious—some aid to the vision must be sought, some means of learning details which cannot be seen with the naked eye. Here it is, then, that the microscope comes into play, and it is not too much to claim that, besides being the source of additional interest, the instrument is a great educator —that is to say, it trains, without appreciable effort, the hand to be skilful, the eye to appreciate, and the brain to elucidate.

Moreover, without for one moment suggesting that observations in the open air should not be considered the most essential part of Nature study, we must agree with a recent writer in *The Country Home*, that 'there will be times when the most enthusiastic Nature student cannot be out of doors—long, dark winter evenings and wet days even in summer, when indoor work must take the place of outdoor. It is then that work with the microscope will prove such a fascinating hobby, supplementing, as it does, the observations made with the naked eye, and leading us into regions where it is impossible to travel without it.'

The lowest forms of plant-life are unicellular, and often extremely small. The microscope reveals to us that they have powers of locomotion; it shows us also, for instance, that the green colouring on trees and fences which shows after rain is made up of myriads of minute plants, taking in gases from the air and earth-salts from the surfaces on which they live, and making their food in the same way as the cabbage or the oak-tree. The whole science of bacteriology and the discoveries of the minute fungi which cause disease and putrefaction, which give the taste to butter and the flavour to cheese, entirely depends upon the high powers of the microscope, and though investigations in this direction are not for the young beginner, still, they lie before him when he has mastered the details of his instrument.

The story of the interesting, though for a long time hidden, methods of reproduction among the mosses and ferns have been revealed by the microscope. The eggs and motile fertilizing bodies in the so-called flowering heads of the moss have been discovered, and the determination of the species by the systematist depends to a great extent upon the microscopic details of the capsules which grow from the egg, and are really another generation, producing spores without fertilization and getting its nourishment from the original moss-plant. In the ferns and their allies, however, we find the sexual organs are borne by a tiny plant like a minute liverwort, which springs from the spores on the fern frond when they fall to the ground, and this little plant nourishes the egg as it develops into a new fern, which, unlike the green moss-plant, is the sexual generation.

Fig. 109.—Travelling Microscope Packed in its Case, which Measures 9″ × 3½″ × 4″.
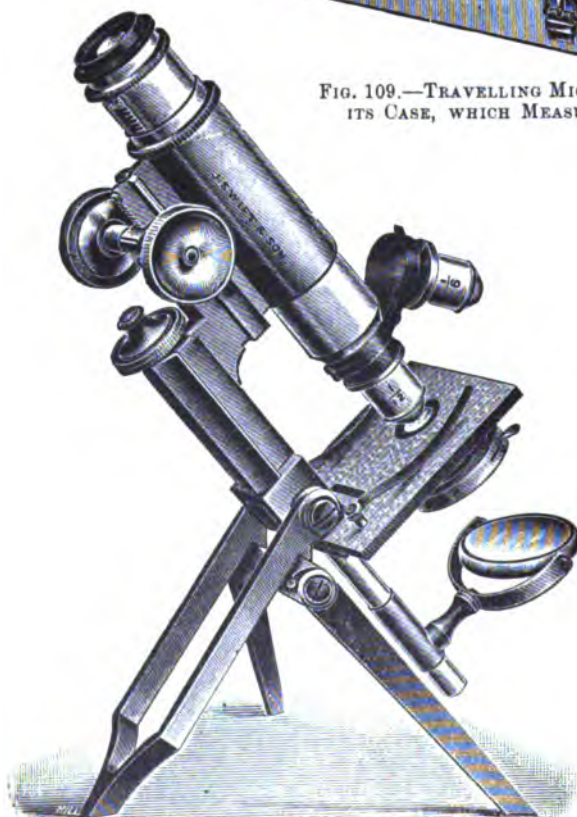
Fig. 110.—Travelling Microscope set up for Use.

Before going on to speak of the use of the microscope in the various branches of natural history, we may point out that it can with advantage be used from time to time to lend an additional interest to ordinary Nature study.

The youngster who sees the pollen of various kinds of flowers as a powder may well be introduced to the variety of shapes and sculpturing which the grains present. Many of the hairs which clothe and protect the commonest plants are fascinating when their details can be seen.

An unfortunate occurrence such as the stinging of a youthful naturalist by wasp or bee may well lead to the examination of the sting of the insect, and possibly the hairs of the nettle, while the delicacy of natural objects compared with those made by man may well be brought home by examining the point of a fine needle with a microscope, and seeing how far more clumsy it is than either of the other two structures to which allusion has been made.

We need not deal further with this side of the question, for those who look for suggestions as to microscopic work can glean them from the paragraphs in which more systematic work is discussed. It may be pointed out here, however, that the microscope may be used by the young student so soon as the informal stage of nature study is passed. The writer can say from personal experience that the interest which can be aroused is very great, while excellent work with the microscope has been done, for instance, by gardening lads who for years have used no instruments of greater precision than spades, and rakes, and hoes.

We need not dwell any longer upon the botanical side, except to say that the whole structure of plant bodies lies before the student. There are all the interesting details to be worked out in connection with the formation and storage of starch grains, which vary in different plants; while one may examine the delicate hairs on the roots which take up water and food materials, or the thread-like fungi which sometimes enter into partnership with the roots and do the work of root-hairs, as in the heaths and rhododendrons, not to mention the bacteria-like organisms which produce nodules on the roots of plants belonging to the pea family, and supply their willing or unwilling hosts with nitrogen which they are able to get from the atmosphere.

Then there is the structure of the fibres which prevent stems from breaking, and of the tubes which carry water with all the various kinds of thickening which strengthen their walls and prevent them from being crushed in as the plant grows and the pressure within the bark increases. There is the bark itself, made up of many empty brick-shaped cells, and the places known to botanists as lenticels, where the bricks are, as it were, heaped together, instead of making a solid wall, so that air can penetrate to the living tissues below. With the microscope we see how the annual rings come to be made in a woody stem. We can learn the structure of a bud and the growing tip, and really come to know how a plant is built up.

If the living plants should pall, we can cut thin slices of fossils and trace the affinities between plants of bygone times and those of the present day. In fact, there is no end to the beauty and the interest that is revealed by the microscope when it is brought to bear on plant structures.

If the material offered by the animal world is not more varied, it is, if possible, even more attractive than that which is to be looked for among plants. To be sure, the botanists have the beautiful flinty shells of the diatoms to study, but among the unicellular animals there is a wealth of forms which are provided with calcareous shells of many shapes, or which build them up with the marvellous discrimination which may exist even in a microscopic speck of protoplasm, from sand grains, or the flinty needles of sponges. It is these shells of foraminifera which, to a large extent, form the ooze which is taken from the very greatest depths of the ocean.

Other slightly higher forms have internal silicious skeletons, and may be caught living in fine nets or their skeletons obtained from deposits such as the Barbados earths, which are largely composed of them.

There are many of the creatures, such as the bell animalcule, the slipper animalcule, and any number of other Infusoria whose conformation appeals to the eye and whose life-histories are fraught with interest. In the sponges our microscope tells us that amongst the supple horny fibres or the delicate needles and geometrically formed spicules which make up the skeleton there are small chambers in which lie the working cells that

differ in practically no respect from Infusoria. A step, however, takes us to the polyps, and a favourite subject for study is the little fresh-water form, with its waving arms, covered with stinging thread-cells that aid it to obtain its prey. Its body, which is all stomach as it were, is lined with cells resembling the proteus animalcules of the ponds, but, unlike them, unendowed with the power of individual locomotion.

For charm of shape and delicacy of construction commend us to the skeletons of the polyps, which live in colonies, to the fixed growths on rocks and sea-shells which at first sight look like delicate seaweed, but which on examination are seen to bear innumerable little cups in which the polyps are seated. Almost microscopic, too, are the small jelly-fish which bud off from these colonies, and by an alternation of generations reproduce not themselves, but colonies like those from which they sprang. Passing over the hedgehog-skinned creatures covered with little nipper-like projections or spines, whose internal structure is of sufficient beauty to repay the trouble of grinding sections, and leaving on one side the wheel animalcules, pretty Polyzoa, and the host of creatures known as worms, which offer many problems to the biologist, we come to the molluscs.

Their shells are usually large enough to see with the naked eye, but there is a great fascination about the structure of their calcareous coverings. Simple though this may be in the bivalves, it is intricate and puzzling enough in the univalves to satisfy the demands of those who wish to exercise their brains and ingenuity. Then the examination of the tongue-like organs of the slugs, and whelks, and limpets is an aid to the classification of these forms. These structures themselves, covered as they are with minute rasp-like teeth, are so very varied and beautiful that the pleasure of examining them, apart from their scientific investigation, can be well understood. The true snails, again, are often provided with minute calcareous spicules of characteristic shape in the various species, called the ' love-darts,' which are useful for classsification purposes, and form beautiful objects for display.

It is when we come to the insects, however, that we meet with even more unlimited material. Some of them, like the tiny fairy-flies—of which, according to the late Mr. Enock, five can

walk abreast through the hole made in a piece of paper with a pin-point—are so small that they have to be examined under the microscope in their entirety, while the parts of other insects show a wealth of detail, and illustrate in a marvellous way the changes which Nature can ring on a single plan.   Take, for instance, the mouth-organs of the cockroach.   At first sight they little resemble those of the bee, which sucks rather than bites, and appear to have no connection with the proboscis of the butterfly, intended merely for drinking up honey.   On careful examination, however, it can easily be seen how the mouth-organs of the two latter have been modified from the first ; and a similar comparison may be made with the stylets of the flea, the piercing organs of the bug, and the lancets of the gnat.

It is only when we examine the wonderful proboscis of the fly that a real difficulty arises.   We may mention also the beautiful scales which give the colours to butterflies, and which resemble those found on the more lowly wingless insects known as spring-tails and bristle-tails, which have never known what it is to fly, and have only survived because, when their relatives took to an aerial life, they were thrown out of competition with them.

There is, indeed, no end to the work which can be done on insects—their breathing apparatus consisting, as it does, of a series of tubes, from which the air is laid on, as it were, all over their bodies ; their beautiful antennæ, and the joints of the legs by which beetles, for instance, are recognized, offer fields for enquiry and objects of interest of which the student will never tire.   The dexterous dissector will find full scope for his powers when unravelling the organs of insects, and finding out how these are equally well adapted to the requirements in the smaller forms of life which possess them, as are those of the higher animals, whose general anatomy it needs no microscope to elucidate.

In the vertebrates, as in the case of all living things, the minute structure must be learnt from the microscope, and, though the sections are more troublesome to obtain than in the case of vegetable tissues, there are a host of things that can be examined and worked out.   Among them we may mention the scales of fishes, the hairs of animals, the feathers of birds. Everyone can see for himself how the feather is built up ; and,

although all the larger feathers are made on the same plan, there are differences in detail.   The various birds, indeed, might occupy the attention of a lifetime.

It is not needful to dwell any further on the question of the use of the microscope to the student of Nature, or what lies before those who decide to take advantage of it.   In conclusion, one may say that it should be part of everyone's education to learn how to use a microscope, and to have some knowledge of the minute details of the living world.

POND LIFE, WITH SPECIAL REFERENCE TO THE ROTIFERA *

By THE LATE C. F. ROUSSELET, F.R.M.S.

Methods of Collecting, Preliminary Examination, and Keeping.

THE fascinating study, under the microscope, of the living microscopic objects found in ponds, canals, and lakes, collectively known as 'pond-life,' requires, first of all, that you should catch your game. The object of this note, therefore, is to discuss those methods of collecting which, with a good many years' experience, has proved to me the most practical, efficient, and time-saving; it is intended for the young naturalist or beginner who desires to make the personal acquaintance of these minute atoms of life, and thereby gain a better understanding of all living things.

A few of the principal localities for collecting pond-life in and near London may be mentioned. The nearest and most convenient available piece of water is the Grand Junction and Regent's Canal, which runs from east to west, on the northern side of London, from Victoria Park to Hanwell, and is readily approachable wherever access can be gained to the towing-path. Wimbledon Common and all the great parks have a lake, such as Victoria Park, Regent's Park, Hyde Park, Richmond Park, etc., which all afford good collecting-grounds. Smaller ponds are found in abundance in fields and commons in and beyond suburban London, and I need only mention a few such places: Epping Forest, Higham Park, Hadley Wood, Totteridge,

* Originally published in *Knowledge*, and reproduced by permission.

PLATE VII.

FIG. 111.—A TYPICAL GROUP OF POND LIFE ORGANISMS.
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Hampstead Heath, Ealing Common, Hampton Court, and Putney Common.

Apparatus Required.—A few pieces of apparatus are indispensable, and these are the following:

1. A Queketter's collecting-stick with ring-net and bottle, and cutting-hook (see p. 88).

2. A flat bottle.

3. A pocket magnifier.

4. A hand-bag with sundry wide-mouthed bottles.

The collecting-stick can be obtained from most opticians. It is a hollow walking-stick with an inner rod to increase its length when required, and provided with a screw at the end for the attachment of either ring-net, dipping-bottle, or hook.
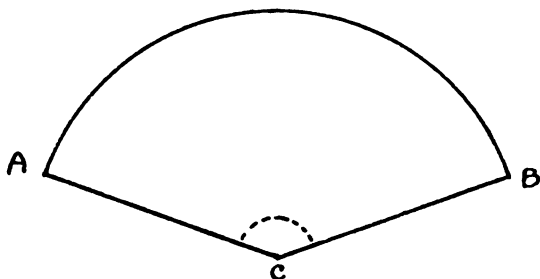
FIG. 112.

A, C = 9 in. ; angle at C = 140°.

The ring is a stout brass hoop, about 6 inches in diameter. The net, which is sewn on to the ring, is made cone-shaped, about 6½ inches long, and at its apex is tied a small, rimmed tube of clear glass, about 3 inches long by 1 inch wide (Fig. 113). The material of the net should be either fine muslin, known as 'soft mull,' with meshes fine enough to prevent the Infusoria and Rotifera going through, and yet allowing the water to run out freely, or else a silk material known as 'Swiss bolting silk,' used by millers for sifting the various grades of flour, and obtainable from all mill furnishers; No. 16 of this silk material has the required fineness.

The net is most important, and some care should be taken to have it properly made. Allowing a margin for the seam and for sewing round the ring, the shape and dimensions of the material for a 6-inch ring should be as represented in Fig. 112. This will

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give a net slightly larger than is required, but as the material is sure to shrink a little, it will be of the right size after having been used once or twice.

The cutting-hook is a curved knife which can also be screwed on to the collecting-stick, and is intended for cutting roots or water weeds which are otherwise out of reach.

The flat bottle can be obtained from opticians, well made, and the parts joined by fusing with fusible cement (Fig. 113). When invented by the late Mr. T. D. Hardy, it was made by cutting sides and bottom in one piece out of stout india-rubber or similar material, 4 to 5 inches long, by 2 to 2½ inches wide, and ⅝ to ¾ inch thick; a square of thin plate-glass of same size, cemented by means of Miller's caoutchouc cement on each side, completed the bottle. A thick piece of india-rubber is, however, so expensive that it is cheaper to buy the finished article. The flat bottle is used for searching over pond-weeds with the pocket lens at the side of the pond, or examining the water which has been collected and condensed with the net. In round bottles it is very difficult to see minute animals clearly, whilst a thin flat bottle allows the whole contents to be readily scrutinized with a pocket lens of considerable power, and one can at once determine whether it is worth while to take home a sample from that particular pond for further examination under the microscope.

The pocket magnifier best adapted for field-work is an aplanatic lens, magnifying six diameters, which has a very large flat field, long focus, and perfect definition all over the field.

Method of Collecting.—The various groups of plants and animals known as 'pond-life,' found in fresh-water lakes, ponds, and ditches, consist of Algæ, Desmids, Rhizopoda, Infusoria, Sponges, Hydras, Rotifera, Polyzoa, Cladocera or Water-fleas, Copepods, Hydrachnida, Worms, and Insect larvæ. These can be divided for the purpose of collecting into two groups—the free-swimming, and those that are usually attached to water-plants or submerged objects, and each of these groups must be captured in different ways.

All free-swimming or floating forms, which collectively are designated by the word 'plankton,' are best secured with the net. The net is passed through the water two or three or more

times, and then held up; the water will run out in half a minute and quite at the last the condensed animals will be seen entering the little bottle like a cloud, where they can be subjected to a pond-side examination. It is best, however, to empty the contents into the flat bottle, in which the examination with the pocket lens becomes very much easier, and most of the forms one is acquainted with can be recognized at a glance. In this way thousands of Algæ, Infusoria, Rotifera, Daphnia, etc., can be captured in a few minutes if the pond be a prolific one. Having thus ascertained that the dip contains some desirable forms, the water is poured into a large, wide-mouthed collecting bottle, of which three to six should be carried in the bag. These bottles should be numbered; for it is often advantageous to keep the water of different ponds separate, so as to be able to know at home from which pond a particular creature has come. Ponds vary exceedingly as regards their contents in pond-life; a small pond may be very prolific, whilst another, possibly a larger piece of water only a few yards off, may contain hardly anything worth collecting. By trying all the different ponds, small and large, within reach of an afternoon's walk, one usually succeeds in obtaining a good gathering of free-swimming forms. The net quickly condenses a large volume of water, so that few species, even if present in small numbers only, will escape being captured. Several other methods of condensing pond-water have been devised, but the collecting-net with bottle attached is so simple and effective that we need not trouble about any other apparatus. It may be advisable to try the larger ponds in various places, and both near the surface and also in deep water, as some plankton forms may have collected in one particular corner of the pond and be absent elsewhere; this is often the case with *Volvox globator*. The use of a boat on larger lakes is very desirable when available. For rotifers and other active free-swimmers it is not desirable to disturb the mud at the bottom of the pond, but certain species of Cladocera, Hydrachnida, and insect larvæ can only be found at or near the bottom.

The group of attached forms of pond-life comprise such Infusoria as Carchesium, Epistylis, Zoothamnium, Stentor, etc.; Hydra; all Polyzoa and Sponges. In searching for these forms,

a quantity of pond-weeds, or rootlets, are brought on shore with the cutting-hook, and selecting some likely-looking, fairly clean branches, but not the newest growth, one twig after another is placed in the flat bottle in clean water, where it can be examined from both sides with great ease, both with the naked eye and the pocket lens. The tree-like Vorticella colonies—Epistylis, Zoothamnium, Carchesium; the trumpet-shaped Stentors; the Crown Rotifer Stephanoceros; the tubes of Melicerta and Limnias; the various Polyzoa; also Hydra and Sponges, and many others, can at once be seen when present, and in this way good branches can be selected and placed in a separate wide-mouthed collecting-bottle containing clean pond-water. A little experience will soon teach one which branches are likely to prove prolific. As a general rule one may say that old-looking, but still sound and green, branches are the best. The Water Milfoil (Myriophyllum) is one of the best water-plants to examine and collect on account of the ease with which its leaves can subsequently be placed under the microscope. Anacharis is more troublesome, but it is occasionally found covered with pond-life, and is an excellent weed for the aeration of aquaria.

The rootlets of reeds and of trees growing near the edge of the water should be examined for Sponges and Polyzoa, such as Lophopus, Plumatella, Fredericella, etc. In order to obtain some weeds growing near the middle of a pond or lake, a loaded three-pronged hook, attached to a line, may be used; this is swung round, and may be thrown to a distance of 20 to 25 yards, where it sinks, and the weeds that are caught by the hooks are dragged on shore.

By these various means a good collection of pond organisms can readily be made after a little practice. Though the spring and autumn are perhaps the best seasons for collecting, pond-life is never absent, even in the winter under the ice.

Having thus filled some bottles with condensed water from various ponds, and placed some promising branches of water-plants in another bottle filled with uncondensed and clean pond-water, the 'bag' is taken home. It is a great mistake, however, to overstock the bottles with weeds, as the plants in such crowded bottles may begin to decompose, killing most of the animals in a short time.

Preliminary Examination.—On reaching home, the first thing to do is to empty the collecting-bottles into small aquaria, so that the captures may be critically examined, isolated, and, if found desirable, placed under the microscope. By far the best and most convenient way of doing this is to transfer the contents of each bottle into a small window aquarium, filling it up with tap-water. The weeds and rootlets that have been brought home are put in another window aquarium in clean pond-water.

These small window aquaria, with flat and parallel sides 6 to 8 inches long by 5 to 6 inches high, and only 1¼ inches wide inside, are the best nurseries for the microscope. The difficulty of seeing and capturing small objects in a large or ordinary round aquarium is very great, and the use of the pocket lens almost hopeless, whilst in these flat and narrow aquaria no object is out of reach of the lens, and the whole contents can be looked over without difficulty and in a very short time.

By placing the tank on a whatnot at a convenient height before a window, or before a lamp at night, most of the free-swimming rotifers will collect against the glass nearest to the light, where they can be examined with the greatest ease and picked up with the pipette if desired. A disc of black cardboard placed some little distance behind produces a very good dark ground, against which the smallest visible specks stand out well.

The condensed pond-water is, of course, frequently so dirty with floating particles of débris that it is at first hardly possible to see through it; but after standing half an hour it will be found that most non-living particles will have fallen to the bottom, and after several hours the water will be quite clear and every living creature will be readily seen.

During the summer months, when Daphnia and Cyclops are abundant, the net frequently collects these in such numbers that they become a nuisance. In order to separate them, when such is the case, I have adopted the plan of passing the water through a small sieve made of material with meshes sufficiently wide to allow the largest Rotifers and Infusoria to go through, whilst keeping back most of the Cyclops and Water-fleas; the latter are then transferred to a separate tank to be examined by themselves.

It is very desirable to examine the collected objects as soon as

convenient, certainly not later than the day after their capture, as many organisms soon die and disappear under the crowded and unnatural conditions in which they are kept in captivity.

Keeping.—Rotifers in aquaria can often, particularly in cool or cold weather, be kept for a week or fortnight, and some species, such as Melicerta, occasionally for months if food material in the shape of fresh pond-water can be provided. Failing pond-water, water from hay infusions, which mostly contain quantities of bacteria and minute Infusoria, may be added. The various species of Polyzoa and Sponges can also be kept alive a considerable time by feeding them in a similar way, but Hydras require a fare of Water-fleas if they are to thrive.

For keeping microscope life I have found no difference between large and small aquaria, but the small tanks are the more manageable; the great thing to be attended to is the proper aeration with water-plants, of which Anacharis, Fontenalis, and Valisneria are, perhaps, the best, and not to overstock the tank with either animal or vegetable life. The water need not be changed, but a little fresh pond-water should be added from time to time. Larger animals, such as small fish, water-beetles and snails must be excluded altogether from small tanks, and Polyzoa and Sponges must be kept therein in very moderate quantity and small colonies only.

In order to ensure success it is essential to maintain a proper balance between the animal and the vegetable life, and also to supply fresh food frequently, for microscopical animals no more than the larger beasts can live long without food. To some extent, no doubt, they feed on each other, but in a small aquarium their hunting-ground is very limited and the game soon becomes scarce. Asplanchna can be seen under the microscope to feed on Anuræa, Brachionus, Polyarthra, Triarthra, and other rotifers when it can catch them, and their shells and remains are frequently found in Asplanchna's stomach.

On the whole, the best plan is to go out and collect a fresh supply from time to time, and as often as may be convenient. I may mention that at the middle of January I had many thousands of rotifers in a tank which I collected two days before in the Grand Junction Canal, near Westbourne Park Station. The canal was covered with blocks of ice, and the

time spent near the water did not exceed ten minutes, during which I filled a large bottle with water condensed by means of the ring-net.

Aquarium Microscope.—Everyone who has worked at pond-life will have experienced how awkward it is to pick out a particular animal, the size of which requires the aid of a magnifier for unmistakable identification. In order to have both hands free for this operation, and to keep the lens fixed to a particular spot, I devised some years ago a small aquarium microscope (Fig. 113), which is simply a flat metal arm, jointed in such a
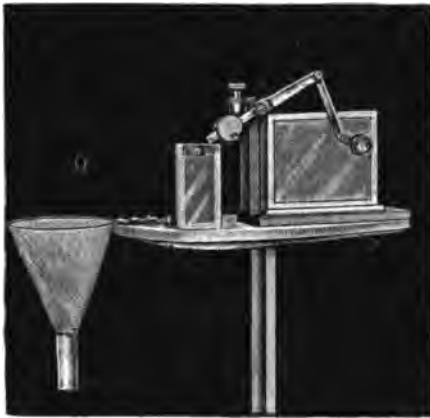
Fig. 113.

way that it allows the lens to be moved all over the surface, but in one plane only, parallel to the side of the window aquarium, whilst the lens is focussed by a small rack and pinion on the left. The whole apparatus is screwed to a small wooden stand, on which the tank is placed. The lens used is an aplanatic combination × 6 diameters, which has working distance enough to focus right through the tank, and sufficient amplification to enable one to recognize most Rotifers, Infusoria, etc., and anything uncommon or new can at once be detected and secured. Moving objects can readily be followed with a lens so mounted, pond-weeds can be searched for anything that may be growing on them, and the lens fixed firmly in any desired position. I have had this tank microscope in constant

use for over twelve years, and can recommend it as thoroughly practical, efficient, and time-saving.

APPARATUS FOR MICROSCOPIC EXAMINATION.

I propose now to describe those methods which long experience has proved to be the most practical in the examination of living objects under the microscope.

Fig. 114 is a photograph of various apparatus used for this purpose, consisting of troughs, pipettes, live-box, and compressor.

After capturing a miscellaneous collection of pond-life and transferring it to a window aquarium placed in front of a window,
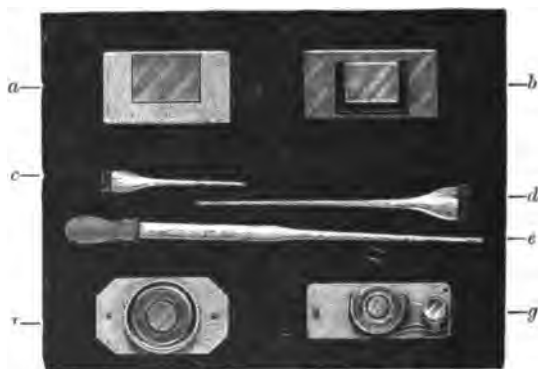
Fig. 114.

as previously explained, it will be desirable first of all to place some of it under a low power of the microscope—say a 2-inch or 1½-inch objective—in order to obtain a better general view of the various animals. The free-swimming forms will mostly have collected on the light side of the aquarium, and can there be picked up quite clean and in vast numbers sometimes, with the pipette e, transferred to a square trough as a or b, and placed under the microscope, where the contents can readily be illuminated from below, both with transmitted light and under dark ground. I prefer to use dark-ground illumination with low powers when searching over the contents of a trough, and when studying the shape, mode of swimming, ways of feeding and living of Polyzoa, Rotifera, and Infusoria. Moreover, the animals scattered through the trough will soon collect in the

spot of light of the condenser, and then the whole field of view will often be a mass of moving, dancing, tumbling, sparkling life.

Troughs.—Pattern a, Fig. 114, 3 in. by $1\frac{1}{2}$ in. and $\frac{5}{16}$ in. thick, is the form I mostly use; it stands upright on the table, is reversible, and can be handled without greasing the well part of the glass. The sides are cemented in the fire by means of a fusible glass cement, and thus the trough is, and remains, watertight. The trough b is also a useful type, but it is not reversible, will not stand by itself on the table, and, being cemented with gold size or marine glue, is liable to leak. The troughs usually sold are semicircular in shape, a very bad type, because, in addition to the above defects, the least amount of tilting on the stage will cause the water to run out over the edge. Thicker troughs are objectionable because the sub-stage condenser cannot work through them, and the animal cannot be properly illuminated, though sometimes such troughs may be required by the size and nature of the object.

Pipettes.—Efficient pipettes are indispensable for serious work. The old way of using the finger on a straight or curved glass tube to capture pond-life is so unsatisfactory that I have been driven to invent new pipettes for more precise and exact work. Fig. 114 c, d, and e, represent the pipettes in constant use; e is a glass tube about $\frac{5}{16}$ inch in diameter and 8 inches long, which tapers from the middle to a point more or less fine, according to the size of the animals one wishes to capture. Over the wide end is placed an india-rubber teat, by means of which any single specimen, or scores of animals, can be sucked up with the least quantity of water; d is another type of pipette, having a still finer action; it is 6 inches long, funnel-shaped at one end, and tapering gradually from the funnel to a fine point; the funnel is $\frac{3}{4}$ inch wide, and covered with an india-rubber membrane; c is a similar, but smaller and finer pipette, $3\frac{1}{2}$ to 4 inches long, for picking up small rotifers in a fraction of a drop of water under the dissecting microscope. The slightest touch on the membrane is sufficient to expel or suck up water, so that one has complete control over the amount that is taken up, and there is much less risk of losing the animal one wishes to transfer to the compressor.

Live-boxes.—The old-fashioned live-box with raised tab', suitable for insects, etc., is quite useless for pond-life, for the simple reason that the objects cannot be properly illuminated with the sub-stage condenser. This consideration led me long ago to design the live-box f, Fig. 114, in which the glass tablet is flush with the brass plate, and is of small size, thus leaving a wide ring all round. This arrangement allows all objects on the tablet to be perfectly illuminated from below by the sub-stage condenser, both with transmitted light and under dark ground, and at the same time they can be reached and followed from above with both low and high powers, even a $\frac{1}{12}$ inch oil immersion, to the very edge of the tablet, and wherever they may wander.

The Compressor.—For more exact work, when it is desired to hold a single animal, and prevent its wandering, I have devised the compressor g, in which the pressure and the thickness of film of water can be accurately regulated by a screw acting against a spiral spring. At the same time, water, or reagents, can be added, if desired, without raising the cover. When properly and well made, this compressor works exceedingly well, and I have had it in constant use for years, but some makers, unfortunately, have introduced variations and so-called 'improvements' which just take away some of the essential and useful points. The semicircular thin cover-glass must be cemented to the under side of the brass ring with a little gold size, so as to be quite firm and rigid, otherwise its action becomes uncertain, and very small objects cannot be held fast, or else are suddenly crushed.

Simple Substitutes for Above.—These are often very useful in an emergency where no live-box or compressor is at hand. An excavated glass slide makes a fair live-cage; a drop of water containing the animals is placed in the cavity so as to just fill it, and no more; another drop of clean water is placed by the side of the cavity, and a clean thin cover-glass is lowered on to that second drop; then, by means of a needle, the cover is slowly pushed across the cavity, which can thus be covered without enclosing an air-bubble; the superfluous water is taken up by blotting-paper, the cover-glass being held in position by capillary attraction. This forms a good slide for low and medium powers, but not for high powers. Another good temporary slide can be made by placing three small fragments of No. 1 thin cover-glass near

the middle of a glass slip in form of a triangle; the drop of
water containing the animals is placed in the centre, and a clean
thin cover-glass is lowered on to the drop so as to rest on the
three glass fragments, which prevent the animals getting crushed.
If there be too much water it can be removed with blotting-paper.
Low and high powers can be used on this slide as far as the
movements of the animals will permit, but not oil immersion
lenses.

Method of Use.—Having detailed the essential apparatus, I
will close this section with a few remarks on the practical use of
same. The free-swimming organisms, including such forms as
Volvox globator, collect on the light side of the window aquarium,
and can there be picked up in small or large numbers, and quite
clean, with the large pipette, and placed in the trough; or any
particular species can be selected with the aid of the tank micro-
scope, and taken up with the smaller pipette, and transferred to
the live-box or compressor in a single drop of clean water, both
hands being free for this operation.

The fixed forms, such as Polyzoa, Stephanoceros, Melicerta,
Floscules, etc., amongst Rotifera, and Stentor, Carchesium,
Zoothamnium, etc., amongst Infusoria, require a little manage-
ment. If simply placed in a trough, these are often obscured or
incapable of being properly illuminated by being too crowded, or
by part of the weed over- or under-lying the objects, and also by
floating particles in the water. The best result is obtained by
trimming—that is, by cutting off a very small piece of weed or
leaf on which the animal is attached—in a watch-glass under a
dissecting microscope, if necessary—and then transferring it with
the pipette to the compressor into a drop of clean water; it can
then be arranged with a needle or bristle as may be desired, and
after lowering the cover-glass, fixed and held fast, at the same
time giving the animal perfect freedom to expand. In this
position the animals can be reached with the sub-stage condenser
from below for transmitted light and dark-ground illumination,
and also with low and high powers, and even oil immersion
objectives from above.

The Microscope.—The Wenham binocular (p. 14) is decidedly
to be preferred to the monocular microscope. Using both eyes,
prolonged work can be undertaken without undue strain, and the

stereoscopic image gives a very much better idea of the true shape of the animals, though the images are not quite so sharp as with the monocular tube ; but this binocular form can immediately be changed into a monocular for high-power work, or whenever desired, by pushing the small prism out of the way.

The binocular is to be used only with the low powers up to the ⅝-inch objective; with higher powers the stereoscopic effect is lost, because the depth of focus, or the plane of distinct vision, is then exceedingly small, and becomes more and more a mere optical section of the object.

A mechanical stage is hardly necessary ; for ordinary work a well-made sliding stage or bar is preferable, and should be provided. Stage-clips, of which opticians are so fond, are abominations, and should be consigned to the dust-bin.

Of illuminating apparatus, the Abbe form of sub-stage condenser, achromatic if possible, is the only one that is really useful for all powers, and that need be considered both for transmitted light and for dark-ground illumination. It should be provided with an iris diaphragm and an arm carrying a central stop; it completely replaces all the older sub-stage apparatus—condenser, spot lens, paraboloid, etc. An illuminant condenser, however, is necessary to render parallel the rays of the lamp-flame, but it should be mounted on the lamp, so as to move with it, once it has been adjusted to project the beam from the edge of the flame on to the flat mirror of the microscope for dark-ground illumination with low powers.

All apparatus used in the examination of pond-life—troughs, live-boxes, compressors, and pipettes—should always be carefully cleaned and dried immediately after use, and in no case should the water be allowed to evaporate in them. Much trouble will be saved by the observation of this rule, and the apparatus will always be ready for use.

PRESERVING AND MOUNTING.

There are few observers of pond-life who have not felt a keen desire to preserve and keep these small highly organized sparks of life instead of letting them die and disappear in a few days. For a close study of this group, well-preserved type

specimens are of the greatest possible assistance and importance, and if such had existed formerly much confusion and inexactitude in their description and classification would have been avoided, particularly in the giving of three or four different names to the same species, which causes so much trouble to the student.

The total absence of type specimens of rotifers to refer to when required led me to attempt the task of working out a method of permanent preservation, and it is now some twenty years since the first successful experiments at preserving them in a fully extended and natural state were made. The method, although so simple now, took fully three years to work out until the right and most suitable narcotic, fixing agent, and preserving fluid were found. By the use of suitable fixing agents not only the external shape of rotifers can be preserved, but also all the internal structure, to the minutest anatomical details, such as the striated muscle fibres, nerve threads, vibratile tags or flame cells, sense hairs, cilia, etc., and frequently important details can be more readily observed than in the living animal.

Narcotizing.—As is well known, no killing agent is sufficiently rapid to prevent the complete retraction of rotifers, and few other animals can contract into such a shapeless mass when we attempt to kill them by ordinary means, such as poisons, alcohol, heat, etc. It is, therefore, necessary to use first a suitable narcotic, which has been discovered in hydrochlorate of cocaine. As a result of many trials, the best solution for most rotifers has been found to be the following mixture:

2 per cent. solution of hydrochlorate of cocaine, 3 parts; alcohol (or methylated spirit), 1 part; water, 6 parts.

Another narcotic which is also very suitable for rotifers is a 1 per cent. watery solution of hydrochloride of eucaine, recommended by Mr. G. T. Harris, for Infusoria and other animals. These narcotics, even so dilute, are not to be used pure, as they would cause the rotifers to contract at once and not expand again. The principle to be followed throughout is to use the narcotic so weak that the animals will not mind it at first, but continue to expand or swim about freely. After a short time its effect will make itself felt on their nervous system, and then some more of the narcotic may be added, until complete

narcotization is produced, or until the animals can be killed without contractings.

But before the operation of narcotizing is begun, it is very necessary to isolate the rotifers in perfectly clean water. The best way is to pick them up under a dissecting microscope by means of a finely-drawn-out pipette, having a funnel-shaped enlargement at the other end, covered with an elastic membrane (Fig. 114 c, p. 232). This pipette forms a most delicate siphon by means of which any selected rotifer can readily be taken up with the least quantity of water, and transferred to another trough or watch-glass full of clean water. This preliminary precaution is necessary, because particles of dirt in the water readily attach themselves to the cilia of dead rotifers, rendering them unsightly under the microscope. Another advisable precaution is to separate the different species, because most species require a slightly different treatment, and because the small species too readily adhere to the cilia of the large species.

Having then isolated a number of free-swimming rotifers in a watch-glass half full of perfectly clean water, one drop of one of the above narcotics is added and well mixed. After five or ten minutes, if the animals continue to swim about freely, another drop is added, and so on until the effect of the narcotic becomes visible, and until the motion of the cilia or the movements of the animals slacken or almost cease, when they are ready for killing. The effect of the narcotic varies very much with different species; some are most sensitive to it, whilst others can stand a considerable quantity for a long time.

Killing and Fixing.—Some practice and patience are certainly required to find out the right time to kill the different species; no general rule can be given, as the time may vary from fifteen minutes to several hours. It is very essential, however, that the rotifers be still living when the killing fluid is added to prevent post-mortem changes in the tissues, which begin at once on the death of the animals.

For killing and fixing several fluids are suitable—namely, $\frac{1}{4}$ per cent. osmic acid, or Flemming's chromo-aceto-osmic fluid, or Hermann's platino-aceto-osmic mixture. On the whole, I now prefer the last named, which gives a finer fixation of the

cellular elements of the tissues and does not stain them so much. It may be explained that the term 'fixing' implies rapid killing and at the same time hardening of the tissues to such an extent as to render them unalterable by washing and subsequent treatment with preserving fluids. Proper fixation is very essential, as no good preservation can be obtained without it.

When the rotifers are narcotized and ready for killing, a single drop of one of the above fixatives is added, and mixed with the water in the watch-glass. A few minutes is sufficient for fixing small creatures like these, and then they must be removed again by means of the pipette to several changes of clean water to get rid of the acid, otherwise they will become more or less blackened. When dealing with marine rotifers, sea-water must be used for washing out, for the difference in density between fresh and sea water is sufficient to cause swelling by osmosis, and the consequent spoiling of the specimen. After thoroughly washing, the rotifers are transferred to a preserving fluid, the density of which does not materially differ from that of water. The best preserving fluid found so far is a 2½ per cent. solution of formalin, which is made by mixing 2½ c.c. of the commercial 40 per cent. formaldehyde with 37½ c.c. of water, and then filtering.

The above are general directions according to which the great majority of rotifers can be preserved. When under the narcotic, the animals must be watched until it is seen that they can swim but feebly, when, as a rule, they will be ready for killing. If they contract and do not expand again, it is a proof that the narcotic used is too strong, and it must be further diluted. The whole method undoubtedly requires great care, and is a delicate operation, which must be performed under some kind of dissecting microscope, but by following the directions here given, and with some perseverance, anyone can learn to prepare a large number of species of rotifers. I would advise that a beginning should be made with some such forms as Brachionus, Anuræa, Synchæta, Asplanchna, Hydatina, Triarthra, and Polyarthra, which are easy, and, moreover, occur, and can, as a rule, be collected in large numbers. A few genera, however, are exceptionally difficult. These are Stephanoceros, Floscules,

Philodina, Rotifera, and Adineta, and it will be better to leave these until considerable experience in dealing with the others has been acquired.

It will have been noticed that the rotifers must always remain submerged in a watery fluid, and be transferred in a drop by means of the pipette. Fluids of lesser density than water, such as alcohol, as well as fluids of greater density, such as glycerine, are unsuitable because they set up strong diffusion-currents by osmosis, which cause the animals either to swell or to shrivel up completely.

Some species of rotifers, such as Triarthra, Polyarthra, Pedalion, Mastigocerca, etc., have an outer surface which is strongly water-repellent, and when these come in contact with the surface film of the fluid even for an instant it is most difficult to submerge them again, and, as a rule, they are lost and spoiled.

Having then successfully narcotized, killed, and fixed the rotifers fully extended, and finally transferred them into 2½ per cent. formalin, the animals may be kept in little bottles, or mounted in the same fluids on micro-slides, either in excavated cells or shallow cement cells.

Mounting.—In the cell of an excavated slip, place a drop of the formalin solution, then transfer the prepared rotifers into this drop with the pipette, and examine under the dissecting microscope to see that no particle of foreign matter has been introduced. Then place another drop of the fluid on the slide by the side of the cell, lower the cleaned cover-glass on that drop, and push the cover cautiously and gradually over the cavity. The superabundant fluid is removed with blotting-paper, and the slide closed by tipping damar gold size cement all round the edge with a fine brush.

The permanent closing of these cells has been a matter of very considerable difficulty. As the result of the experience gained, it is recommended that the cells be closed first with a coat of a varnish consisting of two-thirds damar in benzole and one-third gold size, then two coats of pure shellac dissolved in alcohol, and finally four to six coats of pure gold size. Each layer of cement must be allowed to dry thoroughly well; three days for each layer is not too long.

By the method described above, I accumulated within the period of ten years, 1902 to 1912, a collection of over 500 slides containing nearly 300 different species of rotifers, probably the only collection of the kind in existence, which is of the greatest use for the identification of species and for the general study of this interesting class.

Entomostraca should be narcotized with the same solution as used for Rotifera, then killed with a ¼ per cent. solution of osmic acid, and mounted in a 2½ per cent. solution of formalin.

COLLECTOR'S CALENDAR*

January.

January being the most severe month of the year, lakes and ponds are often frozen over or difficult to approach. Microscopic pond-life, though less abundant than in the spring and autumn, is, nevertheless, nearly always present, even under ice many inches thick. All the following species of rotifers have been taken in January in and near London; but no doubt a great many more could be found by systematic search : *Asplanchna Brightwellii* and *priodonta; Anuræa aculeata* and *cochlearis; Brachionus pala* and *angularis; Notholca scapha; Euchlanis deflexa* and *hyalina; Rotifer macrurus* and *vulgaris; Polyarthra platyptera; Synchæta pectinata, tremula,* and *oblonga; Conochilus unicornis; Cœlopus porcellus; Diaschiza lacinulata* and *ventripes; Proales decipiens* and *petromyzon; Diglena forcipata; Dinocharis pocillum; Monostyla cornuta; Colurus caudatus; Melicerta ringens; Limnias ceratophylli; Œcistes crystallinus; Floscularia cornuta;* and *Stephanoceros eichhornii.* Diaptomus and Cyclops and their larvæ are abundant, whilst Water-fleas are almost absent. Aquatic vegetation having died down, the fixed forms of rotifers and Infusoria should be looked for on the rootlets of trees growing near the edge of the water. Floscules and Melicerta were once found covering such rootlets very thickly. January seems to be the time when the males of Stephanoceros and other tube-dwellers are found, and their presence is often

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* For particulars of the Quekett Club excursions for the purpose of collecting, see p. 88.

betrayed by the thick-shelled, fertilized, resting eggs in some of the tubes, and numerous smaller male eggs in others.

February.

In the early part of the year, when the weather is still cold and ponds are covered with ice, some Infusoria may be found in abundance, particularly the various species of Vorticella—*Carchesium polypinum, Zoothamnium arbuscula, Epistylis flavicans* —attached to submerged rootlets.

Rotifera to be looked for in lakes and ponds, particularly duck-ponds: *Anuræa aculeata, Anuræa cochlearis, Asplanchna priodonta* and *Brightwellii, Notholca scapha, Polyarthra platyptera, Euchlanis deflexa, Synchæta tremula*. The water-plants having mostly died down, the following fixed forms are found attached on Anacharis, or on submerged rootlets of plants, or on trees growing near the edge of ponds and lakes: *Melicerta ringens, Limnias ceratophylli, Stephanoceros eichhornii, Floscularia cornuta*, and other species, *Œcistes crystallinus* and other species.

March.

The same species as those mentioned for February are still to be found, but in greater abundance. Some new Infusoria will have made their appearance, such as *Stentor polymorphus*, which will be found covering the rootlets of Duckweed and other submerged plants, *Peridinium tabulatum* and the free-swimming colonies of *Synura uvella*, etc. Then the very minute and beautiful colonies of Collared Monads, *Codosiga umbellata*, and other species of this group may be looked for, attached to the stems of Vorticella trees.

All the Rotifera forming the winter fauna will become very abundant in March, and as the food-supply in minute Algæ and Infusoria increases, fresh species make their appearance with every rise of temperature. The following additional species may be looked for: *Brachionus angularis; Notholca acuminata, spinifera*, and *labis; Euchlanis oropha; Dinocharis pocillum; Diaschiza lacinulata; Proales decipiens* and *petromyzon; Monostyla cornuta; Diglena forcipata; Rotifer vulgaris*.

April.

All species of Infusoria and Rotifera mentioned as occurring in March are likely to become more abundant in April, which is one of the best months for collecting. The ponds are full of water, whilst they have become approachable, and Daphnias and Cyclops have not yet crowded out the rotifers, as sometimes occurs later on. *Volvox globator* may be looked for, together with the little parasitic rotifer, *Proales parasitica,* inside the green spheres.

Of larger Infusoria, *Bursaria truncatella, Chœnia teres, Amphileptus gigas,* and *flagellatus* will be found, and, of course, crowds of *Euglena viridis.*

Of Rotifera, *Synchæta pectinata* will be abundant, and *Asplanchna priodonta* and *Brightwellii* will have made their appearance in larger lakes and canals; also *Brachionus pala, quadratus,* and *Bakeri; Euchlanis triquetra* and *hyalina; Triarthra longiseta, Diaschiza semiaperta; Rhinops vitrea, Pterodina patina, Mastigocerca bicornis,* and many others.

May.

All the various pond organisms that die down in winter and in various ways produce protected germs to tide over this, for them, unsuitable season, will now have come to life again and begin to multiply at an increasing rate. Many kinds of Desmids should be found in shallow, mossy pools, or along the edge of rivulets. Among Protophyta and Protozoa the green spheres of *Volvox globator* will be found in many localities more or less abundantly, and the various kinds of Acineta should be looked for in quiet, undisturbed waters, where many kinds of free-swimming Infusoria will also be found.

Of Rotifera there are few species which may not be found in May. At one excursion of the Quekett Club to Totteridge in the middle of May forty different species were obtained. To mention only a few : *Notops brachionus,* one of the most attractive rotifers, will have made its appearance; then various kinds of Anuræa, Asplanchna, Brachionus, Cœlopus, Cathypna, Diaschiza, Euchlanis, Furcularia, Mastigocerca, Metopidia, Pterodina, Synchæta, Scaridium, Stephanops ; also *Stephanoceros eichhornii,* Floscules, Melicerta, and Limnias in abundance.

On rootlets of trees growing near the edge of ponds and lakes will probably be found various kinds of Polyzoa : *Fredericella Sultana*, *Paludicella*, and *Plumatella repens*.

June.

If the months of April and May are abnormally cold, pond organisms which usually make their appearance in May are likely to be retarded, and will only come on in June. There are, however, summer forms which hardly ever occur earlier than June, and the most interesting of these amongst rotifers is *Pedalion mirum*, with its six arthropodous limbs ; *Synchæta stylata*, with its long-spined floating eggs, and *Synchæta grandis*, the largest species of this genus, may also now be looked for in lakes and water reservoirs, as well as the rare free-swimming *Floscularia pelagica*. In the same waters will be found two free-swimming colonies of Vorticella : *Epistylis rotans* and *Zoothamnium limneticum*. In June it often happens that certain water-fleas, Daphnia and Bosmina, also Cyclops and their larvæ, increase to such an extent as to render the existence of free-swimming rotifers almost impossible in these waters, and the latter consequently disappear, though they may have been swarming a few weeks earlier. In ponds, however, where this does not occur, rotifers of many genera may be found, and attached to submerged water-plants *Lacinularia socialis* and *Megalotrocha albo-flavicans* should be looked for, whilst in reedy ponds the free-swimming spheres of *Conochilus volvox* may occur. Mossy pools, in addition to their special rotiferous fauna of Philodina, Callidina, Adineta, Cathypna, Distyla, and Monostyla, will also contain water-bears and shelled Rhizopods, such as Diflugia and Arcella and numerous free-swimming Infusoria. Polyzoa, such as *Plumatella repens*, *Fredericella Sultana*, *Lophopus crystallinus*, and *Cristatella mucedo*, though not common, should be abundant in suitable localities.

July.

Collecting in July is usually not so profitable as one would expect, because as a rule most of the shallow ponds are dried up by this time, or have been reduced to a muddy swamp, and in the others Crustaceans, Cladocera, and Cyclops have multiplied

to such an extent as to leave little room for the more interesting
forms of pond-life.

Pedalion mirum should be looked for in large and small lakes, as
it will probably have greatly increased in numbers. The some-
what rare and very large *Asplanchna amphora* and *ebbesbornii*, as
well as *Asplanchnopus myrmeleo*, are summer forms which occur
at this season. Other rotifers that appear in warm weather are :
*Dinops longipes; Triphyllus lacustris; Notops clavulatus; Scaridium
eudactilotum* and *longicandum;* then the free-swimming *Lacinularia
natans* and *Conochilus volvox;* also the fixed *Lacinularia socialis*
and Megalotrocha, which are found attached to submerged water-
plants. All these are very beautiful objects under the microscope,
but by no means common.

Volvox globator will certainly be found in abundance now in
secluded ponds, and inside the green spheres the little parasitic
rotifer, *Proales parasitica*, should be looked for.

The Polyzoa, mentioned last month, will have become more
abundant where they occur ; undisturbed ornamental lakes and
canals are the best places to find them in.

August.

For the collector of Cyclops, Diaptomus, Water-fleas, and
aquatic insect larvæ, August is a very capital month ; not so,
however, for the collector of the more interesting Infusoria and
Rotifera, which are usually quite crowded out by the more
vigorous Crustaceans in the few remaining ponds and pools not
wholly dried up. In larger lakes, however, it is possible to find
occasionally a number of interesting forms, particularly free-
swimming rotifers, such as *Asplanchna priodonta* and *Brightwellii*,
Synchæta pectinata, and the rarer summer forms, *Synchæta
stylata* and *grandis*. Where a ' green ' pond can be found full
of the flagellate Infusorian *Euglena viridis*, there are usually
present also a number of rotifers, such as *Hydatina scnta*,
Eosphora aurita, Diglena biraphis, etc., feeding on the Euglena.

In shady forest pools, overgrown with Sphagnum, quite a
peculiar fauna of moss-haunting rotifers will be found, particu-
larly various species of Callidina, Distyla, Metopidia, Cathypna,
in addition to numerous interesting Rhizopods with shells of
various forms. In similar ponds the large but very rare rotifer,

Copeus spicatus, should be looked for. Of other rotifers that may be met with in lakes, more or less abundantly, the following can be mentioned : *Brachionus pala; Anuræa aculeata, brevispina* and *hypelasma; Dinocharis pocillum; Euchlanis triquetra, hyalina,* and *oropha; Mastigocerca bicornis, elongata,* and *stylata; Polyarthra platyptera; Synchæta tremula* and *oblonga; Pedalion mirum,* and many others.

September.

In normal years many of the dried-up ponds begin to fill up again in September, and become then most prolific in infusorian and rotiferous life, because the disturbing Crustaceans, Cyclops, and Cladocera have been to a large extent eliminated. But also in larger ponds and lakes, which do not dry up, the Crustaceans decrease in numbers and give the Rotifera and Infusoria a fresh chance of increase. The following free-swimming forms may often be collected in immense numbers : *Asplanchna priodonta, intermedia,* and *Brightwellii; Triarthra longiseta; Polyarthra platyptera; Synchæta pectinata, tremula,* and *oblonga; Anuræa aculeata* and *cochlearis; Brachionus angularis; Pedalion mirum; Conschilus unicornis,* and the much rarer *Floscularia pelagica.* Of the fixed forms, *Limnias ceratophylli* and *annulatus, Cephalosiphon limnias, Lacinularia socialis, Melicerta ringens* and *conifera* should be looked for on submerged water-plants, such as Anacharis, Ceratophyllum, Nitella, and on the rootlets of Duckweed. Polyzoa such as Plumatella, Lophopus, Cristatella, should be found in abundance in disused canals and backwaters of rivers and the larger lakes, from which they may be dredged with a loaded hook and line.

It may be taken as a general rule that all the more interesting forms of pond-life become more abundant in September, provided only that the weather is not too hot, but tempered by repeated showers to fill the dried-up ponds with a fresh supply of rain-water.

October.

October is one of the best months for the pond-hunter ; the weather is cooler, the ponds have become filled with rain-water again, with plenty of food material in the shape of flagellate Infusoria, and the Crustaceans are on the decline. In this

month the greatest variety in species of Rotifera is usually found, particularly of the smaller and rarer kinds, and not infrequently thirty to forty species may be obtained in one or two small ponds. As a general rule one cannot expect much variety when a few species are present in excessive abundance. The following is a list of forty-four species of rotifers actually collected on one occasion in three ponds on October 15, 1898, showing what may be looked for :

Floscularia regalis, ornata, cornuta, ambigua, edentata, and *annulata; Limnias annulatus, var. granulosus; Œcistes crystallinus; Philodina megalotrocha; Rotifer vulgaris; Synchæta tremula* and *oblonga; Asplanchna priodonta; Notops hyptopus; Polyarthra platyptera; Eosphora aurita; Furcularia longiseta, sterea,* and *forficula; Proales felis; Diglena biraphis; Mastigocerca rattus* and *bicornis; Cœlopus porcellus* and *tenuior; Rattulus bicornis; Diaschiza exigua; Distyla flexilis; Monostyla lunaris; Dinocharis pocillum; Stephanops lamellaris; Cathypna luna; Euchlanis oropha; Metopidia acuminata; Brachionus angularis* and *Bakeri; Pompholyx sulcata; Notholca labis* and *scapha; Anuræa aculeata, cochlearis, tecta, hypelasma,* and *stipitata.*

On the other hand, the various kinds of rotifers known as summer forms will now have disappeared. *Pedalion mirum* is such a form, which may occasionally still be seen during a warm October, but is then usually very scarce or absent.

November.

With the advent of November, pond-life all round becomes less abundant, and fewer species are to be met with. By degrees many of the water-plants die down, and the fauna is reduced to such forms as can subsist through the winter. Those animals which cannot do this, such as Polyzoa, Daphnia, some Rotifera, etc., have by this time produced so-called winter eggs or resting germs. The winter fauna, however, is much more numerous than is usually assumed. Among rotifers, several species of Synchæta—*S. pectinata, tremula,* and *oblonga*—seem to like the winter as well as the summer: *Asplanchna priodonta, Anuræa aculeata, Polyarthra platyptera, Rotifer vulgaris, Euchlanis deflexa, Triarthra longiseta, Brachionus angularis, Conochilus unicornis, Diglena forcipata, Diaschiza lacinulata*

and *ramphigera*, *Dinocharis tetractis*, and others. Among the
Infusoria, the Vorticella in particular seem to like the cold
season, and a number of different species, and often large
colonies can be found attached to submerged rootlets of trees
growing near the edge of the water. Attached to the fine stems
of Carchesium, Zoothamnium, and other stalked colonies of
Vorticella, the very much more minute but beautiful colonies
of Collared Monads, Codosiga, etc,, are often found, and deserve
careful examination with the higher powers.

In canals and lakes where Cristatella has been abundant
during the summer, their spiny stadoblasts may now be found
liberated and often in large masses floating near the edge of the
water which lies opposite to the direction of the prevailing wind.
These should be collected and placed in a jar full of water with
some Anacharis in a warm room at home, where they will
hatch by the end of December or January, and the beautiful
young Polyzoa can be seen emerging from their box-shaped
prison.

December.

Severe weather in this country does not, as a rule, set in in
December, and the lakes and ponds are not usually frozen over
in the early part of the month. The winter fauna has now
become more pronounced, but includes quite a number of
Infusorians, Rotifers, and Crustaceans. The following species of
rotifers have been collected in December in lakes and canals in
and round London, some of them in great abundance: *Anuræa
aculeata* and *cochlearis*, *Asplanchna Brightwellii* and *priodonta*,
Brachionus angularis, *Diaschiza semiaperta*, *Euchlanis deflexa*,
Melicerta ringens, *Œcistes crystallinus*, *Limnias ceratophylli*,
Floscularia cornuta, *Synchæta pectinata* and *tremula*, *Conochilus
unicornis*, *Rotifer vulgaris* and *macrurus*, *Polyarthra platyptera*,
Notholca scapha, *Triarthra longiseta*. Of Crustaceans, *Diaptomus
castor* and various Cyclops and their larvæ are abundant, whilst
Water-fleas die down. A minute red flagellate Infusorian often
seems to form the chief food material of the above lake fauna.

PLATE VIII.

BRITISH HYDRACARINA.
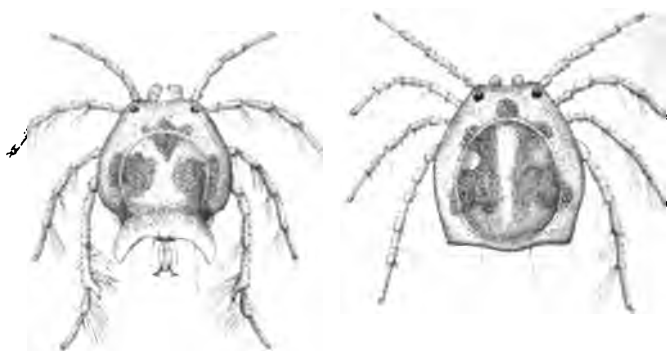
FIG. 115.—ARRHENURUS BRUZELII. FIG. 116.—ARRHENURUS BRUZELII.
(KOEN.) DORSAL SURFACE ♂. (KOEN.) DORSAL SURFACE ♀.
Drawn from specimens found at Wroxham Broad.

FIG. 117.—ATURUS INTERMEDIUS. FIG. 118.—ATURUS INTERMEDIUS.
(PROTZ.) DORSAL SURFACE ♂. (PROTZ.) DORSAL SURFACE ♀.
Drawn from specimens taken by Mr. Hulbert, Wicklow, Ireland.
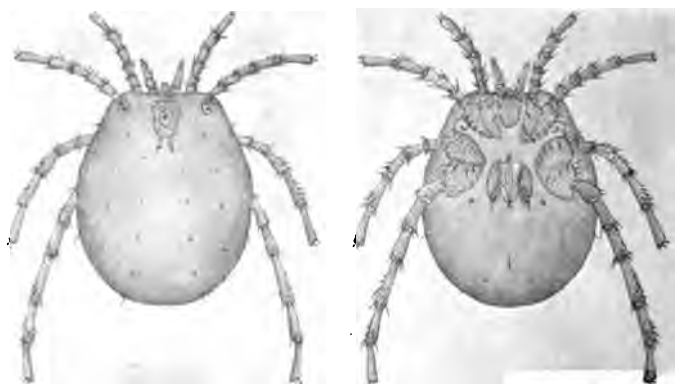
FIG 119.—THYAS VIGILANS. (PIER.) FIG. 120.—THYAS VIGILANS. (PIER.)
DORSAL SURFACE ♀. VENTRAL SURFACE ♀.
Drawn from a specimen taken on Sunningdale Common.

THE COLLECTION, EXAMINATION, AND PRESERVATION OF MITES FOUND IN FRESH WATER

By C. D. SOAR, F.L.S., F.R.M.S.

ANYONE with a love for natural history wishing for a hobby for his spare time would find the study of fresh-water mites (Hydrachnidæ) an extremely interesting one. For variety and beauty in colour, and for differences in form and structure, they are not to be surpassed by any other organisms found in fresh water. Wherever there is a pond, ditch, or stream, the collector is nearly sure of being rewarded for his search by finding one or more species of these interesting creatures. They are easily caught, and can be seen with the naked eye ; they are, however, very seldom recognized without the aid of the microscope. They can be kept alive for a considerable period at home, and are easily preserved when killed.

Life-History.—The life-history of these creatures is so imperfectly known that there is wide scope for an observant naturalist. Although the life-histories of some species have been fairly investigated, the number of such is very limited compared with the species known, and the variety of species which have been recorded in Great Britain are behind the recorded collections of Germany and elsewhere.

These creatures are caught in three distinct stages—the larval, the nymph, and the imago. In the larval stage they are very small, and only have six legs. When they first emerge from the egg they are free-swimming, but they soon become attached as parasites to some other form of pond-life. They will often be found hanging like small red pear-shaped appendages on a great

number of aquatic insects. In many cases the six legs they started life with disappear after they have become firmly attached by their mouth-organs to their host, and they spend the remainder of this period of their existence without any.

This stage is succeeded by the nymph ; the little creatures are then much larger and have eight legs. During this term of their existence they are free-swimming, and can be caught in the net in numbers, but it is impossible to distinguish the sexes.

In the last stage—the adult or imago—all the structure and form are present, but many may be taken that are not fully developed. In the majority of species, the male can be distinguished from the female and the specific differences recognized ; but there are some in which the sexes are so much alike that it is almost impossible to tell one from the other. In others, again, the sexes are so different—as, for instance, in the Arrhenuri—that one would be disinclined to think they could be of the same species.

The three figures (Plate IX.) are intended to convey to the beginner the three stages. Fig. 122 is the larva of *Piona longipalpis*. Fig. 121 the nymph and adult of *Hydrachna globosa* (Geer), showing the ventral surface and the epimeral plates to which the eight legs are attached. Fig. 123 is the larva of an Hydrachnid, parasitic on *Dytiscus marginalis* and *Nepa cinerea*.

There is another point in the adult stage to which it will be well to draw attention. When the mite has first made its appearance from the inert period it spends between the nymph and adult stage, the hard and chitinous parts appear to be nearly fully developed, but the soft parts are not so. The body often appears very small, while the palpi, legs, and epimera, etc., are very large in proportion ; it is also very poor in colour. It would be well to ascertain that the mites are quite developed before making drawings and taking measurements. In my ignorance, when I first began the study of water-mites, I had to discard a number of drawings I had made of different specimens because they afterwards proved to be only different stages of growth of the same species of mite.

Collecting.—For collecting there is no better apparatus than the collecting-stick used by pond-hunters, having a metal ring attachable at its end which carries a cone-shaped net made

PLATE IX.

FIG. 121.—LARVA, NYMPH, AND ADULT HYDRACHNA GLOBOSO. (GEER.)

Note.—All three figures reprinted from the Journal of the Quekett Microscopical Club, by permission.

FIG. 123. — SHOWING LARVÆ OF AN HYDRACHNID PARASITE ON DYTISCUS MARGINALIS AND NEPA CINEREA.

h, Dytiscus marginalis, showing parasites on ventral surface and leg; i, dorsal surface of Nepa cinerea; j, ventral surface of ditto; k, larval parasite detached.
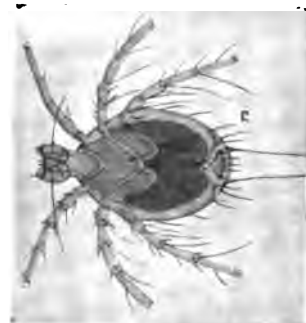
FIG. 122.—LARVA OF PIONA LONGIPALPIS. (KREN.)

of silk or muslin, with a glass tube at the bottom. The advantage of the tube is that the contents can be examined with a pocket lens at any moment to ascertain if anything has been secured worth preserving (see p. 225 ; also Fig. 118, p. 231).

It is advisable to carry as many bottles as the number of ponds that are likely to be visited ; careful record should be kept of the exact locality where each mite is found, with the date of capture, and this cannot be done if all the specimens are carried home in one bottle.

The most convenient way of carrying collecting-bottles is by sewing two strips of thick cloth together with loops of the required size in the same manner as a cartridge bandolier. Such a device can be rolled and stood at the bottom of a bag, and obviates the chance of the bottles breaking by contact.

During the summer months it will generally be found that the most successful captures are made near the edges and in shallow parts of ponds ; in the winter-time the mites get into deeper water. Some mites are to be found only on the mud at the bottoms of ponds, others on the leaves and stems of water-plants. In collecting, therefore, it is necessary to let the edge of the net just skim over the surface of the mud and sand, and up and down the stalks and stems of likely plants.

The under surfaces of leaves should also be scraped with the edge of the net. Anacharis is a very favourite plant of water-mites, and wherever this is found it is almost certain that mites will be secured.

In addition to the free-swimming mites, there are a large number of parasitic forms, and it is as well to examine all forms of insect-life before discarding material. Fresh-water mussels, in particular, also the large water-snails and water-beetles, are specially to be recommended for examination, and once more let me emphasize that if anything is found, notes should be made of dates, places, and general details of the captures.

Examination.—On reaching home the contents of the bottle should be emptied into a porcelain photographic dish, when it will be noticed that the mites generally swim in the corners or along the sides, and can then be removed with a pipette to a large tube filled with clean water in which some Anacharis is placed. This latter will keep the water clean and fresh for a considerable time.

Experience will dictate which species can safely be kept together, a matter in which some discrimination is required, because some varieties prey on others—such, for instance, as Limnesia on Eulais.

Undoubtedly the best plan is to proceed with the examination at once, because a great part of the brilliancy of colouring is lost in a short time, and the mites are much more lively when freshly caught than subsequently. I have, however, kept mites alive in a tube 4 inches by 1 inch, by adding fresh water to replace that evaporated, for a period of twelve months.

The best method of examination is to place the mite on a 3-inch by 1-inch glass slip, turning the specimen on the ventral or dorsal side as may be required, and having every part extended. A cover-glass is then laid over the specimen, and sufficient clean water is allowed to flow between the cover-glass and the slip to fill the intervening space. The specimen may move its limbs and palpi for a short time, but soon becomes quite passive, the weight of the cover-glass being sufficient to retain the body of the mite in position. The slip is then laid on a piece of white card on the stage of the microscope, and illuminated by reflected light; a 1½-inch objective will usually be found the most suitable.

The advantage of this arrangement is that the specimen can be reversed, and both sides examined, and by having an aperture in the cardboard, a further examination may be made by transmitted light. In this latter condition the hairs and claws can be seen very distinctly, particularly if the light be thrown a little obliquely. After examination the specimens can be returned to the tube, and are usually none the worse.

Preservation.—To preserve the specimens they should be placed in the following solution :

10 parts glycerine, 10 parts distilled water, 3 parts citric acid, 3 parts pure spirit.

They can be placed in the solution alive, and although at first the limbs will be contracted, they subsequently relax. It also preserves the colours of hard-skinned mites fairly well.

If at any time it is desired to make a mounted preparation of any mites preserved in this way, they can be transferred to cells containing the same solution. If required for balsam mounts,

the glycerine can be removed by repeated soaking in absolute alcohol, subsequently passing them through clove oil.

It will be found that balsam-mounted specimens will have a tendency to vaporize; this can be obviated by making a small hole in the body of the mite in a position which is of no consequence, and thus allowing the balsam to penetrate. I think the soft-skinned mites mount best in glycerine solution; I do not mount in this medium myself, but have some beautiful preparations by Mr. Taverner, in which the construction is shown to the best advantage. They have been in my possession for some time, and show no signs of deterioration.

Should any readers take up the study of these beautiful creatures, date of collecting, localities where discovered, and particulars of anything they may have observed new in the life-history, particularly varieties of colouring, should be carefully kept, together· with, if possible, drawings. There is one mite, *Piona rufa Koch*, which has been found in England in three distinct, bright, and beautiful colours—viz., red, green, and brown.

COLLECTING AND PREPARING FORAMINIFERA*

By ARTHUR EARLAND, F.R.M.S.

THE Foraminifera, in spite of their beauty, the important part which they have played in the building up of our earth, and the many interesting features of their life-history, have not met with so much favour among microscopists as many groups of far less importance. This comparative neglect is largely due to mistaken ideas as to the difficulty of obtaining and preparing suitable material, and it is proposed to show, so far as possible within brief limits, that the collection of material is within the reach of every visitor to the seaside, and that the subsequent preparation presents no unusual difficulty to the microscopist.

The chief sources from which Foraminifera may be obtained are :

1. Dredged material, including anchor muds and sands.

2. Shore gatherings made between tide marks.

3. Sands, clays, and limestones of various geological ages, especially from cretaceous and tertiary deposits.

As probably very few readers will have the opportunity of dredging for material, and as anchor muds, which often contain an abundance of shallow-water forms, are rarely obtainable, owing to the strange reluctance of seamen to lend themselves to the collection of scientific material, it is not proposed to enter at any length into the methods of collection by means of the dredge. The method of preparation for materials of this class is essentially the same as that for shore gatherings.

Dredging.—The ordinary naturalist's dredge can be used for the purpose of collecting foraminiferous material from the sea-

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* Reprinted from *Knowledge* (with additions).

PLATE X.

BRITISH FORAMINIFERA.

FIG. 124.—MASSILINA SECANS.
(d'Orbigny.)

FIG. 125.—TEXTULARIA AGGLUTINANS.
(d'Orbigny.)

FIG. 126.—BULIMINA, LAGENA,
NODOSARIA.

FIG. 127.—NODOSARIA OBLIQUA.
(Linné.)

FIG. 128.—PULVINULINA AURICULA.
(Fichtel and Moll.)

FIG. 129.—POLYSTOMELLA CRISPA.
(Linné.)
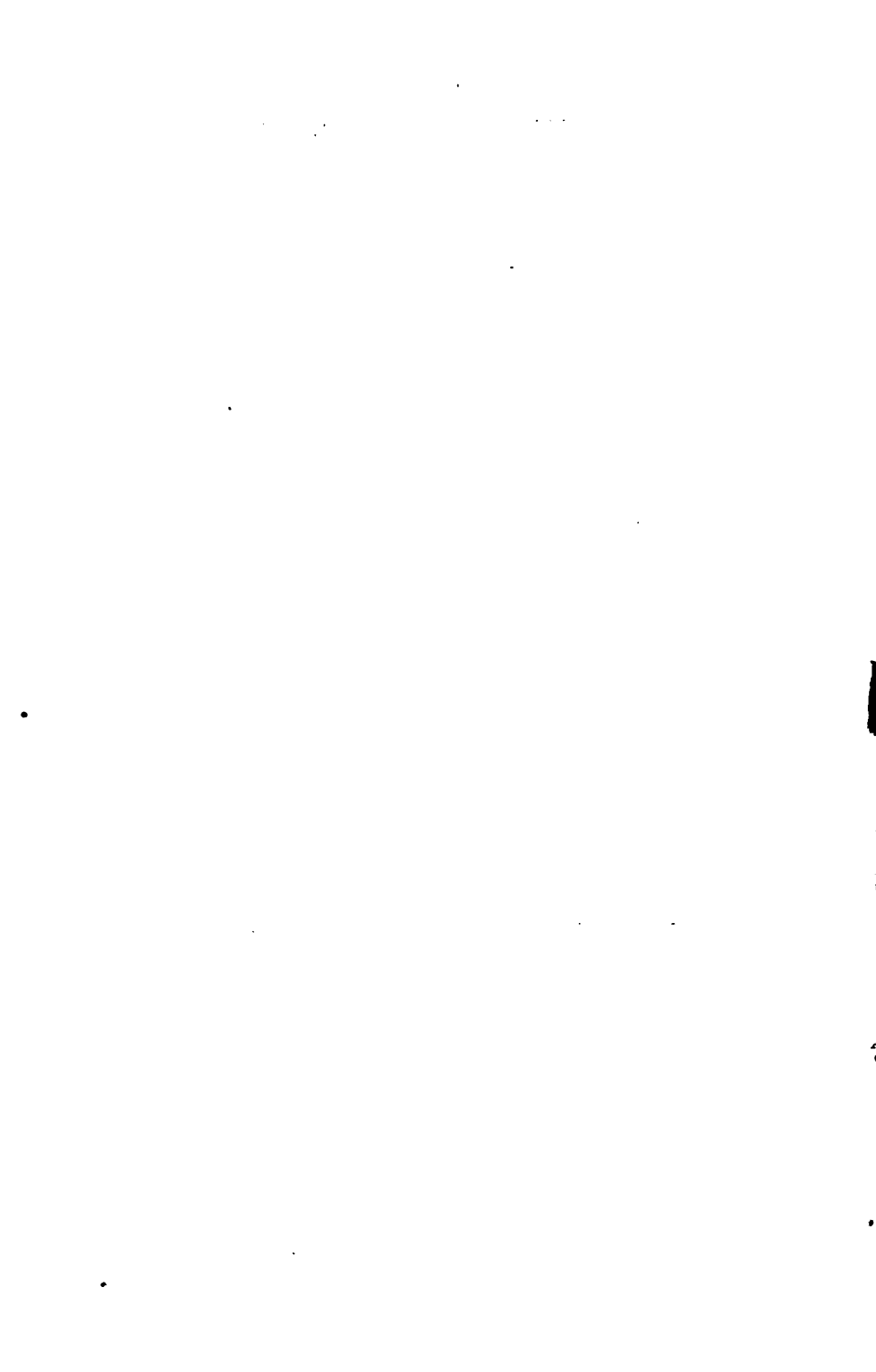
bottom, but if the dredge is of the usual type, with the bag made of rope-net, it will be necessary to insert a canvas lining in the lower end of the bag, in order to ensure the retention of some of the finer sand and mud.   The size of the canvas bag must be governed by the strength of the dredge and the power of the lifting gear.   A large deep-sea dredge such as is used on scientific cruises will have a mouth 4 or 5 feet in width and a bag 6 feet long.   Such a dredge on a soft bottom will fill up in a quarter of an hour or less, and as it weighs a ton or more, the lifting gear must be correspondingly powerful.   When the dredge is operated and lifted entirely by hand, quite a small bag in the end of the small naturalist's dredge will be as much as can be managed.

After the dredge has been emptied on board, the material is usually washed in a large tub through a series of sieves from ½ to ⅛ inch mesh, in order to separate the Mollusca and other large organisms.   A supply of the mud *as dredged* should first be set aside in a canvas bag and labelled ' Original deposit.'   This would be required for any quantitative analysis of the organisms contained in the mud, many of which would be lost during the washing process.

After selecting the softer organisms left on the various sieves for preservation in alcohol or formalin, the residue can be transferred to separate canvas bags and labelled with particulars of the sieve from which it was obtained.   Large species of Foraminifera, and especially arenaceous types, will be found retained on the ⅛-inch sieve.   The coarser sieves will as a rule contain only stones and molluscan or echinoderm fragments, but these will often be found to be covered with sessile Foraminifera.

The tub in which the material has been washed will now be found to contain a bottom deposit of mud and sand and a large quantity of muddy water.   As a rule, this muddy water contains an abundance of the smaller species of Foraminifera in suspension, and as it takes a long time for them to settle down (in rough weather the motion of the ship will keep them constantly in suspension) the muddy water should be baled off and strained through a fine silk net, such as a tow-net.   By this means many species may be obtained in abundance which would otherwise escape observation.   After the water has drained away through the net, the fine mud may be preserved in a bag, or,

preferably, in alcohol. Formalin, having an acid reaction, should never be used for the preservation of Foraminifera.

The bottom deposit of mud from the tub should then be preserved in canvas bags for preparation ashore. If the bags are thoroughly, but slowly, dried over the engine-room, and stored in a dry place, the material can be preserved for many years uncleaned and without deterioration, although it is better and easier to clean it as soon after collection as possible.

Shore Collecting.—The apparatus required is of the simplest character, and consists of a scraper for removing the surface film of sand, which alone contains Foraminifera, a spoon for scraping material from ripple marks and depressions, and a metal box, or canvas bag, to contain the gathering. The best scraper is a thin plate of celloidin (about the thickness of a visiting card), such as a 'photographic' film, as the thinness and flexibility of this material enables the collector to make his scraping with less admixture of sand than is possible with the glass or metal slip usually recommended.

Thus equipped, the collector sallies forth between the tides. Probably everyone has noticed when at the seaside the white lines which run along the sands parallel with the retreating tide. A pocket lens shows that the white material consists largely of the minute shells of Foraminifera, of which some are of a lustrous white colour, due to the comparative abundance of the Miliolidæ —a family of common occurrence in shore gatherings, characterized by opaque shells of a milky white or 'porcellaneous' texture—while others are more or less glassy and transparent. These 'hyaline' forms are much less noticeable to the naked eye. They are mixed in varying proportions with fragments of shell substance—ostracode shells, cinders, and the lighter debris of the shore—and their presence in these lines is due to the separating action of the water, which on a smaller scale we shall later on employ in the cleaning of our collected material. The rocking action of the wave on the extreme edge of the ebbing tide keeps these shells and fragments of light specific gravity in suspension until after the heavier sand-grains have subsided, and so they are left behind in the ripple marks and depressions of the sand. Sometimes a local eddy of the tide, produced by the neighbourhood of a projecting rock, or of groins and piers, causes

the material to be gathered together in large quantities, which show as extensive white patches on the sand, and prove a real gold mine to the collector, who will then obtain more material in half an hour than he could gather in several days from the ripple marks.

The collector must not conclude that there are no Foraminifera present because there are no white patches to be seen, but, remembering the way in which these patches are formed of the lighter débris of the shore, must look for Foraminifera wherever he observes that such débris has been deposited.

In the English Channel and many other shores approximate to steamer routes, coal-dust and ashes are thrown up in such quantities as to mask, more or less entirely, the white Foraminifera, and the ripple marks and patches on the sand may be black in colour.

On every coast, at intervals of varying distance, there are spots which appear to be the foci of the local tides and currents, and here the material will be found in the greatest abundance. These points will soon be discovered, and may be worked at every tide, but they vary continually with the set of the tide and wind, so that a spot which has proved rich may be quite bare the next year. Thus, in October, 1896, Bognor—always a rich collecting-ground—had its richest point to the west of the pier ; while in September, 1901, there was very little material obtainable except at Felpham, two miles to the east, where the beach was thick with débris.

Having found the material, the collection is quite an easy matter. With the celluloid scraper at an angle of 60°, the thin surface film of Foraminifera and débris is easily scraped into a heap, and transferred to the box or bag. Great care must be exercised not to dig down into the sand, for nothing but a heavy bag will result from this, the Foraminifera being confined to the surface layer. The material thus collected may be either cleaned at once, or, after being slowly dried—avoiding great heat—may be packed away in bottles for a more convenient period.

Cleaning.—The apparatus required for the preparation of the dried material is simple and inexpensive, and, if desired, much of it may be easily improvised. The most necessary articles are a photographic developing-dish of china, quarter-

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or half-plate size according to fancy, sieves of different sizes and materials according to the collector's pocket, a cylindrical glass jar with a lip, and without any neck or constriction at the top, or a large plain glass jug, and a retort stand or tripod, made of an iron ring riveted on three legs.

The sieves can be made by any coppersmith, and it is very convenient to have a series of varying degrees of coarseness; but, for the beginner, two sieves of 40 and 120 meshes to the inch respectively will be sufficient. The writer's sieves are of copper, 4 inches high, 4 inches diameter at top, sloping to 3 inches diameter at the bottom. A smaller size, made of telescope-tubing $1\frac{1}{2}$ inches in diameter and 1 inch deep, is very useful for washing small gatherings. The writer also uses Mr. Heron-Allen's method of serial sieves fitted one within another. But great care is required in the use of this method, as the lowest sieves, having the finest mesh, speedily become choked with material, and then overflow, causing the loss of specimens. Zinc, which is cheaper than copper, can be used for the sieves.

The wire gauze, which can be obtained from any large iron-monger, varies in price according to the number of meshes to the inch. The finest obtainable has 120 meshes, the diameter of each aperture being about $\frac{1}{300}$ inch. If a finer sieve than this is required, as it sometimes may be, the size of the aperture may be reduced by silver-plating the gauze, or, preferably, by the use of silk bolting cloth, which may be obtained up to 200 meshes to the inch. The wire gauze must be strained tightly over the sieve and soldered neatly to the edge, so that there is no ledge of solder inside to retain unwashed material. If silk is used, a sieve must be made without a bottom, and having a turned-back edge at the lower end, so that the silk may be strained across and secured with string or a rubber band. Very useful and effective sieves can be made out of brass tube 3 inches diameter, having a detachable clamping collar operated by a screw. The silk is strained over the tube and held in position by the collar. They are much easier to clean than the usual sieve, but are expensive to make. The most useful sizes for a series of sieves are, in my opinion, 12, 20, 40, 80, 120, and 150 (silk) meshes to the inch.

Before cleaning the shore material, it must be slowly and thoroughly dried. It should then be passed through the twelve-mesh sieve to remove all the coarse débris, stones, shells, cinders, etc. Few, if any, of the British shore species, except sessile forms, will be found in this coarse residuum, but it should be looked over with a pocket lens for these, or for abnormally large specimens. In some dredged materials and in tropical gatherings, however, this coarse residuum will be found to be full of Foraminifera.

The material which has passed through the twelve-mesh sieve consists of Foraminifera mixed with other light débris and a considerable quantity of sand, and the collector must now proceed to eliminate the whole, or nearly the whole, of the sand, and as much as possible of the other débris, by means of two operations—'floating' and 'rocking.' If the quantity of material to be operated upon is small, it may be treated off-hand, but if there is much, it is well to sift it out into varying degrees of fineness by passing it through a series of sieves. This will simplify the floating operations by ensuring that the particles are approximately of similar weight.

The floating operations must be performed at a sink, and, if possible, in daylight, the process being more uncertain by artificial light. The finest sieve (120 wire or 150 silk) is thoroughly wetted and rested on the tripod. The glass jar is then filled with water nearly to the brim, and a few spoonfuls of sand slowly poured into it. If the material is coarse the sand sinks instantly, and in the course of a few seconds most of the Foraminifera follow suit. By holding the jar to the light the course of the falling particles can be followed, and at the proper moment a sudden tilt empties the whole of the water and most of the Foraminifera into the sieve, the sand and the heavier 'forams' being left in the jar. The purity of the material in the sieve, which is usually called 'floatings,' will depend upon the skill and judgment of the operator, and is largely a matter of practice. The residuum in the jar must be washed out into a basin for further treatment, and the operation repeated with more sand and water until the whole of the gathering has been treated. The time allowed for subsidence will vary with the fineness of the sand, so that in the case of the finest siftings

nearly a minute may be required. The actual time can only be determined by watching the falling material in a strong light.

In the case of very fine sand, the tension of the surface film of water is so great that the sand grains float almost as readily as the Foraminifera. This difficulty may be overcome by shaking up the contents of the jar, covering up the top with one's hand while so doing.

If it is desired to obtain a very pure gathering the jar should be stirred up and allowed to settle for a few minutes. The lighter species of Foraminifera will be found floating and adhering to the sides of the jar at the surface of the water. They can be removed with a cigarette-paper or feather, and washed off into a separate small sieve. I call these 'double floats.'

The residuum, which had been set aside in a jar, may now be treated by the 'rocking' process for the separation of the remaining Foraminifera. Taking the photographic developing dish (or a zinc tray may be used as a substitute), enough of the residuum is placed in it to cover the bottom to a depth of about $\frac{1}{4}$ inch, and covered with about $\frac{3}{4}$ inch of water. If the dish is then rocked with a combined up and down and circular motion, the Foraminifera will rise in suspension in the water, and by a little careful manipulation may be gathered in one corner of the dish. A sudden tilt will then empty them with the water into a sieve. The operation should be repeated with two or three lots of water, and the material left in the dish will then be found to consist almost entirely of sand. The material left in this second sieve, known as 'washings,' is not so pure as the 'floatings,' for it contains a large percentage of broken forms and shell fragments, coal-dust, and other débris. It may be further purified, if desired, by being dried and 'floated' once or twice in the glass jar.

If the floatings thus obtained contain much animal or vegetable matter, as is sometimes the case, it is advisable to boil them in a very weak solution of caustic potash. This will not damage the Foraminifera so long as the boiling is not carried on too long, and it effectually removes the animal matter, which otherwise would encourage fungoid growths. This process must, however, be used with great caution if the gathering contains arenaceous Foraminifera. All trace of the caustic potash must be removed by frequent washing.

The processes already described are intended for recent *sandy* gatherings. When the material is in the form of dredged mud, it is first necessary to get rid of the finest particles of this mud, for if the water is turbid it becomes very difficult to judge the right moment for separating the floating forams. The mud should be broken up into small lumps, about an inch cube, and slowly but thoroughly dried. It is then placed in a basin and covered with water, which rapidly breaks it up into a fine mud. Boiling water acts most quickly, and if the mud was very 'sticky' when dredged the addition of a little washing soda will facilitate the cleaning process. Great care must, however, be taken not to expose your silk sieves to the soda solution for more than a few seconds, and to wash them thoroughly afterwards. Such specimens as may be observed floating on the surface of the water may be easily removed by means of cigarette-papers, which are placed on the surface of the water. The forams adhere to the papers, which are then carefully lifted off and dried, the specimens being afterwards brushed off into a tube. Many delicate forms, which would almost certainly be broken in the subsequent processes, may thus be obtained in a perfect state.

The mud remaining in the basin is then washed, a little at a time, by placing it in a sieve of fine silk gauze, through which a gentle stream of water from the tap (or preferably from a 'rose' attached to the tap by a short length of tubing) is kept running until all the fine particles have been removed. The muddy water should be allowed to settle in a bath, and the solid residuum scraped out and thrown away. The sandy residuum left in the sieve should be thoroughly dried, and is then ready for examination under the microscope, or, if desired, it may be further reduced in bulk and purified by the floating and rocking processes already described.

Fossil Foraminifera occur in marine deposits of all geological ages, from the Cambrian to post-tertiary, but they are, as a rule, of sparing occurrence until we reach the cretaceous period. The harder chalks and limestones can only be studied by means of thin sections, but the softer chalks, shales, and clays may be broken up by drying the material in small pieces and washing it over a fine sieve in the manner just described. Floatings are seldom procurable from fossil deposits, owing to the weight of

the specimens, which are generally more or less infiltrated with pyrites or other mineral matter.

Some chalks and shales which resist the disintegrating action of water after being dried may be broken up by the action of a crystallizing salt, which has been absorbed in a fluid state. Acetate of soda has the most rapid action, but very fair results may be obtained with common washing soda. The material, after being broken up into small pellets, is dropped into a boiling saturated solution of the salt, and kept at this temperature for a short time to allow of penetration. The salt is then allowed to cool, and in cooling crystallizes, the formation of the crystals breaking up the outer layer of the material. On being warmed, the soda dissolves again in its own water of crystallization, and the crystallization is repeated over and over again until the lumps are broken up. The resulting mud is then washed in the ordinary way.

The best Foraminifera from the chalk are those obtained from the interior cavities of hollow flints. They are often in the most perfect state of preservation, and the chalk in these cavities being of a powdery nature, they are very easily cleaned.

Cleaned Material should be sifted into varying degrees of fineness, and each grade kept separately in a tightly corked tube, noted with locality, date, and any details as to the species contained in it, which may be likely to be useful for future purposes of reference. If the material has been properly cleaned and dried it can be kept unaltered for an indefinite period, but if put away damp fungoid growth will quickly set in. This can be destroyed and the material sterilized by a prolonged soaking in spirit, the material being afterwards dried once more.

Examination and Selection.—For this purpose a picking-out tray will be necessary. A serviceable tray for a beginner is made by covering a slip of card with coarse black ribbed-silk, the ribs running longitudinally along the slip. A thin wooden ledge must be glued round three sides of the slip to prevent the forams rolling off when the stage of the microscope is inclined at an angle. The material is sprinkled over the slip, and the ridges of silk keep the forams from rolling about. The specimens required can then be easily selected by means of a fine sable brush,

moistened by drawing it between the lips, and transferred to a prepared cell or slip.

For serious work there is nothing to equal the 'Stephenson' model of binocular microscope, which has the double advantage of a flat stage and an 'erect' image (Fig. 37, p. 32). This type I have used for the last twelve years, and, after an equal number of years' work performed under the harassing conditions associated with the 'Wenham' type of binocular and a tilted stage, I appreciate its advantages more every day. A full description of the instrument and its associated apparatus, including much useful information on methods of collecting and mounting, will be found in a small book* written by my friend and collaborator Mr. Edward Heron-Allen, F.R.S.

Mounting.—The best fixative for mounting is gum tragacanth, which is almost invisible when dry, being quite devoid of the objectionable glaze which characterizes gum arabic. It is also much less subject to variations of moisture than gum arabic, which alternately contracts and expands with changes of weather, and often fractures delicate forms. Powdered gum tragacanth should be used in the preparation of the mucilage. Put a small quantity of the powdered gum in a bottle with sufficient spirits of wine to just cover it and shake up. Add a small crystal of thymol or a few drops of clove oil, or oil of cassia, as an antiseptic, then fill the bottle with distilled water and set it by for some hours. The gum will form a thick mucilage, and may be used of varying thicknesses according to the size of the Foraminifera. For most forms it should be of about the consistency of cream, and it may be used liberally in mounting, as it shrinks very much in drying.

The same gum diluted to a watery consistency can be used as a fixative for Foraminifera mounted in balsam. If the slide is thoroughly dried before the balsam is added the gum becomes quite invisible.

For very large and heavy Foraminifera, seccotine or some other liquid glue may be used with advantage, gum not being of sufficient strength to hold them safely.

Internal Casts.—Many fossil Foraminifera and some recent

* 'Prolegomena towards the Study of the Chalk Foraminifera,' by Edward Heron-Allen. London: H. S. Nichols and Co., 8, Soho Square, 1894. 2s.

forms have the internal chambers filled with mineral infiltrations, either glauconite or pyrites. These internal casts reproduce more or less perfectly the shape of the sarcode body of the animal. They may be obtained by decalcifying the specimens with very dilute nitric acid, just faintly acid to the taste, or a saturated solution of picric acid. To obtain perfect casts the process must be carried out very slowly. When decalcification is complete the resulting casts should be carefully removed with a pipette, and deposited in a spot of gum on a slip. They will not stand transference with a brush without damage.

Artificial casts of the animal body may be taken in paraffin wax, by a method suggested by the writer to Mr. H. J. Quilter, and described by him in the *Journal of the Quekett Microscopical Club* for 1903.

Briefly, the method as improved by subsequent experience is as follows : (1) Boil the Foraminifera in weak caustic potash to remove animal matter, wash thoroughly and dry. (2) Soak in chloroform for a few days, until all the air is expelled from chambers. (3) Drop straight from chloroform into melted paraffin wax of high melting-point, and keep the wax hot over a spirit lamp until all the chloroform has been expelled as bubbles. (4) Then remove the Foraminifera with a forceps or brush to cover-glasses, and remove any excess of paraffin wax with blotting-paper. (5) After the wax cools, the foram is attached to the cover by a film of wax. Clean the top surface of specimen by rubbing it with a fragment of blotting-paper dipped in xylol. This operation should be done under the microscope, as it is only desired to remove the surface film of wax. (6) Drop the cover-glass into a beaker of strong nitric acid. The rapid effervescence, set up by the destruction of the calcareous shell, tears the outer film of wax apart, and the internal cast floats free. It may then be taken up carefully on a brush and mounted dry.

The process is a most interesting one, and the results with the larger species are surprisingly beautiful. The smaller species are, however, very difficult to manage satisfactorily.

Sections of Foraminifera may be made, and will be found very useful, and sometimes indispensable, for the study of their internal structure. If it is merely desired to lay the shells open in order to show the interior chambers as an opaque object, this

may be done by fixing them on a slip or cover-glass and rubbing lightly on a fine hone or on a sheet of fine ground glass.   The risk of fracture is greatly lessened if the chambers are first infiltrated with wax or with Canada balsam in the same manner as in the preparation of casts, the matrix being removed with xylol after the section has been made.   If, however, it is desired to cut a transparent section, the chambers must be infiltrated with toughened balsam.   After soaking in chloroform until all air is got rid of, the specimens should be placed in a watch-glass and covered with pure balsam, not balsam dissolved in xylol or chloroform.   This must be left for a day or two, then toughened in a cool oven.   If the balsam is then melted the specimens can be removed to slips, where they will be fixed on cooling, and are then ready to be ground down.   When ground to the required depth the slide is again warmed, the specimen turned over, and the grinding repeated with extreme care until the section is reduced to the required thickness.   The operation is one of the most extreme delicacy, and no microscopist need expect to succeed without preliminary failures innumerable.   A single movement on the hone beyond the requisite point generally suffices to wipe the specimen out of existence.

Still better results can be obtained by the use of gold size instead of Canada balsam, but the toughening process in this method depends on time, and great care is required in the turning of the specimen, as the slightest excess of heat destroys the gold size.

Classification.—The microscopist who takes up this fascinating group will soon wish to know something about the specimens he finds.   Here his difficulties begin, for there are some thousands of species, and the literature of the subject is scattered through as many thousand pamphlets and reports in all languages.   But two works may be mentioned which will suffice to educate the beginner up to the stage of generic identification. ' The Foraminifera: An Introduction to the Study of the Protozoa,' by Frederick Chapman, A.L.S. (Longmans, 1902), is written in simple language and adequately illustrated with rough cuts of type species.   It also contains useful bibliographical lists of the principal papers up to that date.

' The Foraminifera,' by J. J. Lister, F.R.S., in Lankester's

' Treatise on Zoology ' (Second Fascicle, Part I., Introduction and Protozoa ; London, 1903 ; Adam and Charles Black), is a more technical work, but full of information of the highest value. Having mastered these the collector should be able without hesitation to assign his specimens to their correct genera.

Beyond this, there is no royal road to knowledge of species, which can only be attained by degrees. Perhaps the best method at this stage would be to consult the report by H. B. Brady on ' Foraminifera ' (' Zoology,' vol. ix.) in the series of reports on the scientific results of the *Challenger* Expedition. This is obtainable at most Museum Libraries, and figures an enormous number of species. It also contains a full bibliography of the subject to the date of publication (1884), and the student who has made himself familiar with Brady's work and the selected papers recommended by Chapman will be in a fair way to become a specialist on a group which at the present time has, unfortunately, too few workers in the field.

NOTES ON THE COLLECTION, EXAMINATION, AND MOUNTING OF MOSSES AND LIVERWORTS

By T. H. RUSSELL, F.L.S.,

Author of 'Mosses and Liverworts.'

Collection of Specimens.—The appliances for the collection of specimens are simple in the extreme. For many years I have been in the habit of putting the material gathered in the field into old envelopes that have been cut open at the narrow end instead of at the side. Not only do these form most convenient pockets for the purpose, but notes can be made on them at the time, of the date and locality when and where the plants were found, and of the names that suggest themselves on a first inspection (a useful aid to the cultivation of the power to recognize plants in the field). Rough memoranda, too, may be added afterwards of any special features of interest that present themselves on a closer examination of the contents, and that need elucidation. Squares of stout paper will be equally serviceable, and, indeed, some of these should always be included for the putting up of larger plants. They will also prove more convenient when specially wet material has to be stored, as the only objection to the use of the envelopes is that moisture is apt to loosen the gum which fastens the several portions together.

A magnifying-glass with a fairly large field, for making a preliminary acquaintance with the gatherings, and for exploring purposes, will also be required. I always carry a second glass of a higher magnifying power—*e.g.*, a platyscopic lens with a magnification of ten diameters—which is often most helpful when minute details have to be ascertained. An old knife, with which to dislodge the plants and to free them from soil and

grit; a lead-pencil for making notes of habitats, etc.; and a satchel, to serve as a receptacle for the envelopes and papers, both when empty and filled, complete the only real requirements of the moss-hunter.

On reaching home the envelopes that have been used should be placed in a warm room, in an upright position, and with the ends open as widely as possible so as to admit air, and the packets unfolded; this will allow the specimens inside to dry, a matter of no small moment if the risk of mildew is to be avoided. After standing thus for a few days they can be safely put away until a convenient opportunity occurs for examining the contents, when soaking in hot water will speedily restore the plants to their original freshness, though not, of course, to life. The small china saucers, made in different sizes, to be procured from any artists' colourman, are very convenient for this purpose. The ease with which mosses can in this way be revived for examination constitutes, to my mind, one of the chief attractions which this branch of botanical research offers to anyone in search of a hobby, for while the gathering of specimens forms a healthy outdoor occupation for all seasons of the year, and adds immensely to the pleasure and interest of a ramble in the country, their examination and mounting may be deferred for any length of time, and will provide the most pleasurable recreation by the fireside in the long winter evenings.

Preparation of Specimens.—To prepare specimens for examination or for mounting, some form of dissecting microscope is practically a necessity. For many years I used an ordinary magnifying-glass of low power, mounted in a light metal frame, at one end of which is a small collar, which slips over a screw fixed in an upright position in a small metal stand, and provided with a nut by means of which the lens can be adjusted to any required height, and this simple expedient is still often very serviceable.

Another inexpensive and useful instrument for the purpose is the ordinary watchmaker's glass, consisting of a lens set in a deep horn mount, by the help of which it can be retained in position in front of the eye; but care must be taken, especially when this is used in mounting, that the muscles are not unconsciously relaxed in the interest of the work, and the

glass allowed to fall. As a rule, however, I now employ the more modern binocular dissecting microscope, which is also of the greatest assistance in mounting.

For dissecting purposes ordinary sewing needles set in cedar penholders are frequently very handy, and it is well to have one or two bent at an angle to the holder, as these will be invaluable in mounting, both for the purpose of altering the position of objects after the cover-glass has been put on and for removing stray bubbles of air. In order to bend a needle into almost any form, it is only necessary to heat it in a spirit-lamp to a red heat, and then allow it to cool; this will render it soft and pliable. After being bent to the required form, it can be rehardened by plunging it when red-hot into water.

While dissecting instruments of various kinds can be purchased, I know of none that better serve the purpose than such as may easily be constructed by the use of sail needles and glovers' needles (No. 4); the former for ordinary work and the latter for the more delicate operations. Sail needles can be obtained at an ironmonger's and glovers' needles from a draper. The needle, in addition to having a fine point, is ground with three flat faces, which give as many cutting edges. In the case of the sail needles these edges are somewhat blunt, and the needle should therefore be sharpened on a hone before being used. Cheap black-lead pencils make very good handles in which to mount the needles. After being cut into suitable lengths, they should be well soaked in hot water; this melts the glue that fastens the two portions of the wood together, and the lead can then be easily removed. The needle should be placed in the groove thus provided for it, and should be allowed to project some little way beyond the end of the wood. A fine pin or needle is then run through the eye, and is cut off with a pair of wire-nippers, leaving just enough to press into the other half of the holder, when put into place. Some amount of packing may be necessary, especially, with the glovers' needle, to keep the needle rigid. The two parts of the holder are then fastened together with liquid glue or seccotine, each end being afterwards tied round with fine twine, as a still further precaution. It is well to scrape off all traces of the glue from the outside of the holder, as otherwise it is apt to stick unpleasantly to the lips if held there for a minute in the hurry

of mounting. Two pairs of forceps (one with curved ends), a pair of small scissors, a small camel's-hair brush, and one or two small lancets, will practically complete the implements required for all ordinary dissecting, to which must be added, for the purpose of microscopical examination and subsequent mounting, a stock of the usual glass slips (3 inches by 1 inch), and cover-glasses of two or three different sizes. Though the round cover-glass is generally to be recommended, both on the ground of appearance and of ease in sealing, yet with many of the larger mosses the square form will prove more serviceable, as it gives more mounting surface, while for dealing with plants of any considerable size specially large pieces of cover-glass may have to be procured.

Mounting.—Owing to their small size and the facility with which their original freshness can be revived, as already noticed, mosses can be far more satisfactorily preserved than is possible with ordinary flowering plants. The greater number may be readily mounted on the ordinary glass slips, and in this form they not only occupy a comparatively small storage space, but remain, for all practical purposes, as fresh as when they were gathered. I have specimens in my collection now that were put up twenty years and more ago, and which have altered little in appearance in the meantime.

I have tried several materials and compounds for mounting purposes, but unhesitatingly give the palm to glycerine jelly, both on account of the ease with which it may be manipulated and by reason of its admirable preservative powers. I shall, therefore, mainly confine myself to a description of the method of procedure when this medium is used. I have for many years made my own jelly according to the following recipe, which is a slight variation on that given in Carpenter's work on 'The Microscope.' Take 2 ounces (by weight) of the best gelatine, 6 ounces of water (also by weight), and 6 of glycerine. Soak the gelatine in the water until it swells (this takes about forty minutes); then place the vessel containing the gelatine and water (a jam-pot is very serviceable; it should be provided with a cover of some kind) in a saucepan of water, and boil over a slow fire until the gelatine melts. When the gelatine is cool, but still liquid, add the white of one egg, and mix well. Boil the gelatine

as before, until it becomes thick with the coagulated albumin—this takes about twenty minutes; add the glycerine and 25 or 30 drops of carbolic acid and mix well; strain through filter-paper before the fire, and a clear pale yellow jelly should be the result.

The specimen to be mounted must first be cleansed from all earth and grit in water, the spores gently expelled from any capsules, and all air-bubbles removed by means of the dissecting needles, and too much care cannot be given to this somewhat tiresome process, as on its due performance the success of the mount, to a very large extent, depends. But our moss is not yet ready to be put into the jelly, for if this were straightway to be done, the effect would inevitably be that the leaves would curl up beyond recognition. A simple plan for guarding against this is to boil the plant for a few seconds over a spirit-lamp, in a teaspoonful of water, in which three or four drops of glycerine have been mixed; but unless time is of moment, it is better to leave it to soak for twenty-four hours in a mixture composed of water, 1½ ounces (fluid); rectified spirit, 1½ ounces; and glycerine, 5 drachms. The small china pans in which moist water colours are sold are very useful for this purpose. Not unfrequently I subject it to both treatments, as this tends more thoroughly to remove all air. When taken from this preparatory bath care must be taken to remove the fluid adhering to the plant as far as possible; this may be done by placing it on a glass slip, and tilting this so as to allow the superfluous liquid to drain off, with possibly a judicious application of blotting-paper, though this must be used with caution, as otherwise fresh air is apt to be admitted.

A hot-water bath is essential for mounting with glycerine jelly. A simple and inexpensive one can be made by means of a small glass tumbler, provided with a closely fitting tin cap or lid, having a piece cut out of the margin, leaving just room enough to admit the neck of a small glass bottle containing the jelly. The bottle can thus hang in the hot water in the tumbler, when the lid is in place, by means of its projecting lip, which rests on the top of the tin cover, and in this way the jelly is kept melted, and is, moreover, close at hand for use. The advantage of having a *small* bottle for this purpose (which is, of course, replenished from time to time from the larger stock bottle) is that the necessity is obviated of continually remelting the same jelly, which would

cause undue evaporation of some of its component parts. When my mounting is likely to take long I wrap a piece of flannel round the tumbler, in order to retain the heat in the water as long as possible. The glass slip on which the mount is to be made, as also the cover-glass must be first carefully cleaned. A good plan is to rub them over, between the finger and thumb, with acetic acid, in order to remove all traces of grease, and then to wash them in warm water, afterwards drying with a soft cambric handkerchief, a final polish being given with a wash-leather. The glass slip is now placed upon the flat tin cover of the hot-water bath, a small pool of the liquid jelly is put upon it by means of a pipette, and the specimen, after being freed from the preparatory fluid, is gently lowered into the jelly. While the latter is kept liquid by the heat of the water-bath, all air-bubbles must be carefully removed with the dissecting needle, and here the binocular dissecting microscope will be found most helpful.

It is impossible to exaggerate the importance of this extraction of air, for nothing detracts more from the appearance of a mount when viewed under the microscope than the presence of these disfiguring silvery globes, lurking among the delicate leaves, or perhaps in the teeth of the peristome; and my own rule always is that, rather than allow a serious blemish of this kind, the slide must be sacrificed or the mount be recommenced. I have found it a great help in many cases, especially when an object likely to retain air or an undue amount of the preparatory fluid is in hand—as, for instance, a large empty capsule or a plant with the leaves closely covering the stem—to put it into a little jelly on a spare glass slip, and then to extract the air as far as possible before transferring it to the slip on which it is to be mounted. The whole plant thus becomes more or less saturated with the melted jelly, and the air-bubbles cannot find their way back to the mount, as they are apt to do if the whole process is carried out on the one slide. A second hot-water bath is then a practical necessity, in order that the jelly thus left in contact with the specimen may not solidify before the latter is transferred to the glass slip. When everything has been prepared, and the specimen is in place, immersed in plenty of the liquid jelly, the cover-glass is taken up with the forceps and gently lowered on the jelly, beginning from the left-hand side, driving the jelly (and too often,

alas! the specimen also) before it, as it is allowed gradually to fall into place. This is an operation of no little delicacy, as if great care is not taken a large bubble of air will make its way in at the last moment. If, as frequently happens, the putting on of the cover-glass has caused a displacement of the object, this must be rectified before the jelly is allowed to set, and here the bent dissecting needles will be of great service, as a considerable amount of rearrangement can be effected with one of them, and stray air-bubbles may also be removed without disturbing the cover-glass.

Should it happen that not quite enough jelly has been used to fill the whole of the space under the cover-glass, a small additional quantity must be introduced by means of the pipette, care being taken to avoid the formation of air-bubbles. The slide is now taken from the hot water bath and is allowed to cool, and in a few minutes the jelly will have so far solidified that it can be examined under the microscope, when, should any serious defect be disclosed, the jelly must be remelted, and the short-coming be rectified. The final process consists in removing the superfluous jelly from around the cover-glass with a knife, cleaning the slide from all trace of the jelly (a handkerchief moistened at the lips is the most efficient method), and sealing the cover-glass round the margin with some kind of varnish.

I may add that I usually mount two cells on each slide; in the larger of them I place a small portion of the moss, together with a few capsules, if possible in various stages of growth, and two or three perichætial leaves, while the other cell contains some leaves dissected from the plant (where of importance from both stem and branch), and a few pieces cut from the mouth of the capsule, to show the peristome.

It will be very evident that the subject of the removal of air has frequently cropped up in what I have written; this is accounted for by the fact that it betokens the chief difficulty to be encountered in mounting when glycerine jelly is the selected medium. There is, unfortunately, no royal road to success in this particular, and the main thing to rely on is a patient use of the dissecting needles. One or two hints as to matters of detail may, however, be given. Boiling the specimen, more especially if it be old, is often helpful; and soaking it in water that has

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been allowed to *boil* for ten minutes is sometimes recommended. In this connection, too, I may mention that it is important to keep the pipette, used for abstracting the melted jelly from the bottle, quite clean, as, if earthy matter is allowed to get encrusted on it, small bubbles of gas are apt to be formed, and these easily get transferred to the jelly itself.   I have also found that attention to another seemingly trivial point is of no little moment, and that is, when dipping the pipette into the melted jelly, always keep a finger on the open end until the point has reached the bottom of the bottle. As heat naturally causes any bubbles in the jelly to rise to the surface, this minimizes the risk of their entering the pipette, for the lower strata of jelly are, so to speak, tapped.

Glycerine Jelly.—As far as my acquaintance with glycerine jelly goes—and it is one of a good many years' standing—the great objection to its use arises from the fact that after a specimen has been mounted in it, and has stood possibly for years, the jelly may develop an unpleasant tendency to liquefy, as will be evidenced by the presence of small beads of glycerine round the edge of the cover-glass.

While not being able to suggest any unfailing remedy for evils such as this, I have noticed that the observance of a few simple rules will considerably minimize the risk.   These are—

1. Care should be taken to remove the preparatory fluid from the surface of the object as far as possible before mounting, without, of course, running too much risk of admitting air.

2. No pressure should be applied to the cover-glass in order to keep it in position, its own weight being alone sufficient for the purpose.

3. A sufficient quantity of the jelly should be used to allow of a small portion extending on every side beyond the edge of the cover-glass, in order to provide against subsequent shrinkage in the gelatine.

4. The slide should stand for at least two or three months before the additional jelly is removed and the cell sealed.

5. The slides should be kept in a fairly equable temperature, and should not be exposed to draught.

I generally add a very small amount either of carbolic acid, or of a 5 per cent. solution of bichloride of mercury (corrosive

sublimate) to the liquid jelly on the glass slip, before the speci-
men is put into it, as this lessens the chance of any fungoid
growth subsequently developing in the cell.   It will suffice if the
tip of a thin glass rod that has been drawn to a point is just
dipped into the liquid, the small amount clinging to it being
transferred to the jelly, and quickly mixed with it.   In the case
of the bichloride of mercury it is especially important not to
introduce too much, or a slight precipitate of calomel will result,
giving a cloudy appearance to the jelly.

Formalin.—A 3 per cent. solution of formalin is also a most
serviceable mounting medium, more particularly where some of
the more delicate plants are under treatment, though it is, of
course, open to the objections that attach to the use of all liquid
media.   If only leaves are being mounted—and it is for such a
purpose that the solution is most suitable—a cell of some spirit
varnish must first be made by means of a turn-table.   When this
is dry the top of the cell is ringed round with marine glue dis-
solved in benzol (as to which, more hereafter), and sufficient of
the solution is introduced into the cell from a pipette to allow of
its standing well above the walls of the cell.   The object is now
carefully introduced, the cover-glass is taken between the finger
and thumb, and after being brought as near to the solution as
possible, is allowed to fall gently into place.   Should air have
unluckily made its way in, the cover-glass must be quickly raised,
and a little more of the solution be introduced.   The cover-glass
is now gently pressed down on to the top of the cell, and, after
all superfluous moisture has been removed with a handkerchief,
is ringed round with the marine glue solution, and afterwards
with spirit varnish, being finished off, if thought desirable, with
a coat of asphalt varnish.   If a larger object is to be mounted a
deeper cell must, of course, be used.

Varnishes.—I have tried a good many sealing materials, and
on the whole much prefer picture copal varnish (to be obtained
from any artists' colourman) thinned with benzol.   It does not
dry too hard, and in consequence is not liable to crack; while,
should any portion of the object happen to be located near to
the edge of the cover-glass, it can nevertheless be seen through
the practically colourless varnish.   Where the object is of any
size, such as a piece of one of the larger plants, it becomes

advisable to use a sealing medium that will adhere more tenaciously to the sides of the cell, and here nothing serves the purpose better than marine glue dissolved in benzol. I should advise anyone who intends to employ it to get the preparation ready-made rather than attempt its manufacture, for if there is one medium that is more exasperatingly adhesive and sticky than another it is marine glue. It is, however, a very safe material to use, and is, as far as my experience goes, quite free from any tendency to 'run in,' which I have always found to be the shortcoming of gold size. The cell may be finished later with asphalt varnish.

Although I have throughout referred to mosses alone, yet the methods of which I have spoken are equally applicable to the mounting of liverworts. The only additional point to be mentioned with regard to the latter is this: Owing to the fact that the contents of the leaf-cells are specially dense, it is often advisable, in order to render the cell-structure more distinct, to treat the leaves with a strong alkali. The best way is to place a piece of the plant in a few drops of a 7 per cent. solution of liquor potassæ on a glass slip, to cover this with a cover-glass, and then to boil over a spirit-lamp. The plant will need to be well cleansed by boiling in water before the leaves are mounted, and a few of them may then be conveniently included in one of the cells.

CHAPTER XXIII

MYCETOZOA

By A. E. HILTON.

I.

AMONG the byways of Nature there are few pleasanter pursuits than the study of Mycetozoa, an unfamiliar group of obscure organisms which mainly haunt the shady woodlands. For the student with a microscope there can hardly be a more interesting line of research. They are mostly microscopical, often charming, and always suggestive.

As their name implies, Mycetozoa—*i.e.*, ' fungus-animals '—are curiously anomalous in their mode of life. In the upward line of evolutionary progress, their place is at the point where animals and plants diverge ; but they have evolved into neither one nor the other ; they remain dubiously at the parting of the ways, presenting both plant and animal features, the latter preponderating. Botanists and zoologists can alike claim or repudiate them ; but when too little attention is paid to the animal characteristics, and Mycetozoa are regarded simply from a botanical point of view, misinterpretations are the result.

Their propagation, like that of Fungi, is by means of multitudinous, extremely minute, and resistant spores, easily disseminated by wind, insects, and birds ; and they have been found in all parts of the world where looked for. Yet it is not always easy to find them. In searching for specimens, it is well to bear in mind that Mycetozoa can only thrive where fungus and moulds can also flourish, because similar conditions of moisture and decay are essential in both cases. They are therefore likely to be found in somewhat ' mouldy ' situations ; or, to put it less unpleasantly, in those damper localities

' Where the mushroom parasol
Opens to the moonlit rain.'

When first observed, and for long afterwards, Mycetozoa were naturally mistaken for Fungi; and the confusion was perpetuated by the terms 'slime-fungus' and 'slime-moulds,' misleading names which ought to be dropped.

The vital distinction between Mycetozoa and Fungi comes to light in the germination of spores (see Plate XI., Fig. 130). A fungus-spore sends out one or more 'germ-tubes,' which lengthen into hyphæ, and help to form a mycelium for the growth of a new fungus; whereas spores of Mycetozoa each give birth to a free-swimming animalcule known as a 'swarm-spore.' Under the microscope with a ⅛ inch objective, swarm-spores in a drop of water can be seen in rhythmic movement, caused by the lashing of their flagellæ. By means of pseudopodia they capture bacteria, which they ingest as food. After multiplying by repeated divisions, they coalesce in pairs to form 'zygotes,' and these in considerable numbers blend together, forming a 'plasmodium.' This is a free mass of semi-fluid plasm containing the nuclei of the swarm-spores. The swarm-spores lose their individuality, and the nuclei divide and multiply proportionately to the growth of the plasmodium. Some plasmodia are so small or watery-clear as to be indistinguishable in their usually damp surroundings; while others are of larger size and so coloured as to be easily visible to the naked eye. When food is plentiful, and other conditions favourable, they creep about and grow rapidly, generally extending by amœba-like movements in a network of veins over the surfaces of leaves, logs, fallen branches, or other decaying vegetation on which they feed. The orange-coloured plasmodium of *Badhamia utricularis*, creeping over tree-stumps and feeding on the fungus *Stereum hirsutum*, sometimes spreads its lacework over an area of 2 or 3 square feet. In other cases, plasmodia develop out of sight in the interior of rotting wood.

When a small plasmodium, or portion of a larger one, is placed on a sheet of glass on the stage of a microscope, and kept moist in that position by the judicious application of an occasional drop of water, and is viewed by transmitted light through a 1 inch or ½ inch objective, the sight it presents is very striking. Along the principal arteries a current of interior plasm is seen to be flowing, the lacework of the slenderer veins sharing

the circulation, and filling up and spreading fanwise in the direction of general advance, while correspondingly withdrawing in the rear. The streaming commences slowly, gathers speed, flows strongly for a minute or more, gradually comes to a stop, pauses a few moments, and then—reverses! (Plate XI., Fig. 131). All the streamlets now move in the opposite direction; and the process is repeated until the next pause, when the direction is again reversed, and the forward movement resumed. These alternate currents are unequal, the advancing flow being longer and stronger than the retreating flow, so producing the slowly creeping movement of the plasmodium as a whole. It is a fascinating sight to watch, and seems to be unique.

In adverse conditions of falling temperature, dryness, or failing food, a plasmodium may pass into a 'sclerotium' stage, remaining inert and brittle till better times return, when it revives and becomes motile; but this resting-stage is not an essential phase of the life cycle. The foraging of the active plasmodium, extending its network in search of nutriment, and also gathering up in its travels substances which cannot be converted into living plasm, continues until congestion or other obscure causes brings the creeping to an end. At this critical point the distinctively animal phases of the life-history—viz., the aquatic swarm-spore stage and the amœba-like plasmodium stage—reach their climax, the phase which follows being a spore-forming stage, in which the sporangia more or less resemble different kinds of fungi. In no case, however, can the formation of a sporangium of Mycetozoa be compared to the growth of a plant; the process is of quite another nature, and much more rapid.

When the turning-point is reached, plasmodia hidden in the recesses of rotten wood come to the surface, and those which have matured in the open usually find their way to drier situations. The spot where sporangia are found is therefore not often the place where the plasmodium developed, and this explains some seeming anomalies. In the new position, food assimilation ceases, congestion is relieved by the extrusion of all impedimenta, and the whole of the living plasm, rejuvenated by this purgation, divides into a fine dust of generative spores, each spore containing a single nucleus. The eliminated ingredients, consisting of lime, cellulose, and other substances, are deposited

as stalks and substrata, or consolidated into the walls and inner structures of the sporangia; but the capsules are fragile, and, except in sheltered positions, the spores are soon liberated, and scattered by wind and rain, or other means, to fulfil their reproductive functions wherever they find a suitable habitat.   The birth of the swarm-spores brings us round to the point we started from.   The life cycle revolves at no very regular rate; it is largely controlled by surrounding conditions, and is rapid or slow as circumstances favour or retard.   Thus, spores which have been kept dry for several years, germinate in a few hours when placed in water ; and the case is recorded of a ' sclerotium ' which became motile after lying dormant for twenty years !

Many of the smaller sporangia of Mycetozoa are charming objects for low powers of the microscope, if well illuminated to bring out their beauty.   Their variety is surprising, especially in so small a group of comparatively simple organisms.   Taking into account the sensitiveness and fluidity of the plasm during transformation, this is, perhaps, not to be wondered at.   Equally remarkable is the fact that along with so wide a range of variability there is a specific constancy which emphasizes the essential ' unity in diversity ' of living things, even of such plastic creatures as Mycetozoa.

If the student is fortunate in his search for specimens, he may find not only creeping plasmodia and fully developed sporangia, but plasm in the act of passing from the one stage into the other. There is then a rare opportunity for observing the successive phases of form and colour, which are the outward indications of interior rearrangements.   The translucent columnar groups of Stemonitis rising on their stalks, the changing hues of Comatricha and Lamproderma, and the transitional appearances of Badhamia and other species, are transient phenomena delightful to watch.

It frequently happens that both stalked and sessile sporangia are produced by one plasmodium, the presence or absence of a stalk, in such cases, depending upon contingent rather than specific conditions.   A good illustration of stalk formation is furnished by the well-known *Comatricha nigra*, the successive phases of which are shown in Plate XI., Fig. 132.   Stalk and sporangium develop concurrently as the result of one and

the same process, in which there is no analogy to plant growth.

For examination of specimens in the field a pocket-lens is sufficient; but for precise identification the higher powers of the microscope must be used. It is a notable fact that classification of the Mycetozoa is primarily based on colours of spores as seen by white light under a magnification of 500 to 600 diameters, one section of the group comprising species with darker spores, usually violet-brown or purplish, and the other section those with brighter spores of various lighter tints. The living plasm of the spores being nearly colourless, what is seen through the microscope is really the colour of the spore cases, the membranes which rupture and are discarded when the swarm spores escape; but the system of classification founded upon these evanescent envelopes works out sufficiently well to justify the conclusion that the various colours do in some vague way indicate constitutional differences in the spore plasm by which the covering membranes were excreted.

Other features on which classification rests are the presence or absence of lime in the sporangia of the darker-spored species, and the presence or absence in the lighter-spored species of an uniform system of threads or fibrous ' capillitia.' A capillitium, more or less abundant, is usually present in the sporangia interspersed among the spores, only a few species being entirely without it. Both spores and capillitial threads, in many instances, have excessively fine markings upon them, requiring a $\frac{1}{12}$ inch objective to resolve them clearly, and these also are regarded as specific distinctions. Altogether, 50 genera and some 800 species are recognized, about 180 species having so far been found in Britain. A small group, truly; but from the standpoint of elementary biology, very illuminating.

II.

There is mystery as well as charm in the Mycetozoa; but the mystery is often misunderstood. It is futile to continue enquiring whether they are animals or plants, because there is no strict line of demarcation, nor any clear principle on which one can be drawn. All we can say is that in the life cycle animal phases predominate. Beyond that point the question is meaningless.

The real mystery is the problem of survival. Mycetozoa are organisms of a primitive description and world-wide distribution, pointing to very early origin; but we have no record of their remote past, and it is difficult to comprehend how creatures so sensitive and frail can have come down to us in unbroken succession from the earlier ages of life on this planet. The alternative view is that living matter continues to come into being apart from pre-existing living things; but of that we have no proof, so the mystery remains.

After all, the Mycetozoa do but illustrate, in their own peculiar way, the familiar doctrine of the 'immortality of the protists.' Among the lowest forms of life the separate organisms are single cells, which grow to a definite size and then divide. Each of the two daughter-cells repeats the process of growth and division, and this goes on without end, unless the organisms are destroyed by violence or adverse conditions. There is no individual death at any point in the succession; so that, in a physical sense, this is 'conditional immortality.' Similarly, the life phases of Mycetozoa resolve themselves into an endless series of alternating stages in which plasmodia divide into spores, only to recombine into plasmodia which, after growing, divide as before. In the last analysis, 'rhythmical action is the basis of all nature.' There are endless repetitions along all the lines of life, because the mechanism of the universe is periodic, and rhythms unfailingly recur.

III.

In the garden behind my house is a lime tree log, in an advanced state of decay. Most of the bark is broken away; the wood is spongy, and there are deep longitudinal clefts. There are patches of tiny green moss; and fungus, once abundant, still flourishes at times. Spores of Mycetozoa have been scattered upon the log, and there have been good results. During the long drought which now, at the end of a hot summer, is breaking up, Mycetozoa were in abeyance; but heavy rains have brought about a speedy revival. Several patches of *Lamproderma columbinum* have appeared, the sporangia being about ⅛ inch high. They are vertically cylindrical, with black stalks. When first visible on the surface of the wood, the

plasm is in small, watery-white, semi-translucent globules, more or less clustered into little groups. As development proceeds, the sporangia become in turn pinkish, reddish-brown, and black, afterwards drying to a bronze-like or brassy lustre. If a sporangium is placed under the microscope with a ½ inch objective when the white and still translucent plasm is becoming tinted, a capillitium can be seen forming; and the process can be followed until obscured by a surface membrane which eventually renders the sporangium opaque. Mature sporangia of this species, properly illuminated, are brilliant objects for exhibition.

Lycogala epidendrum have likewise emerged in considerable numbers. The æthalia, or compound sporangia, of this species are from ⅛ to ¾ inch in diameter, and at the outset very conspicuous. They are first seen as small globules of plasm of a light orange colour. As they increase in size from continued emergence of the plasm they become pinkish, and in less than twenty-four hours of a bright salmon-pink hue, very vivid and arresting. This slowly changes to a light buff, and finally to a buff colour with a greenish-brown tint. Each æthalium contains vast numbers of light spores, which under high magnification are almost colourless. In groups and singly, 214 æthalia have formed on the log within ten days.

In the spring of last year a curious thing happened. Scattered over a triangular area of about 12 inches on each side numerous patches of light yellow plasm emerged on the higher part of the log, and by cutting into the substratum about ¼ inch it was found to be oozing up from below that depth and from within the substance of the rotting wood. The atmosphere being hot and dry, some water was poured on the log to assist developments, but in three days' time all the plasm had retreated into the wood and disappeared. On the following day it again emerged, but in fresh places closer together; and presently a brilliant light yellow æthalium of *Fuligo septica* had formed, irregularly cushion-shaped, and covering about 9 square inches. Apparently the whole of the original plasm masses were extensions of a single large plasmodium several inches below the surface. The æthalium, encrusted with lime both above and beneath, dried to a dull whitish-yellow, with light

brown blotches.   The spores are small and dark.   A portion of
the plasmodium, taken when it first appeared and examined
under the microscope, exhibited streaming movements, but the
reversing currents took 2½ to 3 minutes each, the plasm, which
was watery-clear, being heavily charged with opaque lime
granules, which seemed to impede the flow.

A few weeks earlier there had appeared on the log a large
patch of crimson sporangia of *Arcyria denudata,* one of the
loveliest of all the Mycetozoa.

IV.

The microscopist who decides to study Mycetozoa starts with
one great advantage.   There is an admirable monograph of the
group.   There is likewise an inexpensive ' Guide to the British
Mycetozoa exhibited in the Department of Botany at the British
Museum (Natural History),'* which contains an excellent intro-
duction to the subject, and is also a handy book of reference to
carry about while looking for specimens.   Much work remains
to be done in the field, not so much to discover new species, as
to understand better the habits of those already known.   There
are still 70 or more kinds of Mycetozoa of which the plasmodia
have either not yet been seen, or have not been identified; and
it is the behaviour of plasmodia under natural conditions which
chiefly requires investigating, so that the life-histories may be
more perfectly apprehended.

The following suggestions, epitomized under their respective
headings, will be useful to beginners:

How to find Mycetozoa.

Requisites.—Pocket-lens.   Strong, sharp pocket-knife for
cutting into wood.   Two or three pasteboard boxes of different
sizes, with thin sheets of cork in bottom, and supply of pins;
so that specimens on leaves, twigs, moss, etc., may be safely
pinned down.   Good boots for marshy places.   Sharp eyes and
patience.

* Both of these works are obtainable at the Natural History Museum,
South Kensington.   The monograph, by A. and G. Lister, is beautifully
illustrated and rather expensive; but copies of the handbook can be had for
1s. each, exclusive of postage.

When to Collect.—All seasons of the year suitable, except during hard frost and prolonged drought. Late autumn and early spring usually best. Warm, misty, close days, after wet weather, should be taken advantage of, especially if thundery conditions prevail.

Where to Search.—Undisturbed woodlands and thickets. Swampy and shady hollows. Old and neglected gardens. Rotting tree-stumps. Old logs, especially if partly submerged. Decaying fir-logs and stumps particularly good. Fallen branches and twigs. Heaps or mats of decaying leaves; turn over slowly with a stick. Among moss. Lower parts of herbaceous stems. Straw heaps, heaps of old straw manure, sawdust, and spent tan. Occasionally found on living trees at some distance from the ground. A plasmodium of Badhamia crept up a hop-pole to a height of 5 feet.

What to Look for.—Jelly-like plasm masses and networks of creeping plasmodia, minute or conspicuous, white, grey, yellow, orange, or other colours. Large æthalia (Fuligo, Reticularia, Lycogala) easily visible at a distance. Patches of large numbers of small sporangia, white, grey, yellow, orange, red, crimson, purple, brown, or black, lustrous or iridescent. Distinguishable from fungi by fragile sporangium walls, powdery spores, and capillitial fibres, unlike general structure of a fungus. Student quickly learns to discriminate.

Care in Conveyance.—All sporangia too frail to stand rough treatment; must be handled gently. If possible, plasmodia and developing sporangia should be studied *in situ*; when that is not convenient, whole or portion should be removed on as large piece of substratum as will go into box, and utmost care taken to avoid shaking or other disturbance; otherwise normal developments arrested. If placed on wet blotting-paper under bell-glass to prevent evaporation, plasmodia may keep active for days, and even for weeks, if supplied with suitable nutriment.

Optical Requirements.

Magnification.—For advanced study of Mycetozoa, including nuclear processes, magnification up to 1,200 diameters is desirable; but less will do to begin with.

Eyepieces and Objectives.—With medium eyepiece, a ½ inch objective and series of lower powers will at first be sufficient. A 2 inch objective for the lowest magnifications is very useful. The gap between can be covered by 1 inch, ½ inch, and ¼ inch objectives, and a limited range of eyepieces. A $\frac{1}{12}$ inch objective can be added later.

Microscope Stand.—Should be of a model giving good working space between stage and tube for examination of larger specimens ; also important in regard to top-light illumination.

Top-Light Illumination.—Not easy to arrange satisfactorily ; unless flooded with light, beauty of sporangia under low powers is largely lost. Light needs to be thrown down on the object at an angle about 30° from the perpendicular—*i.e.*, about 60° from the plane of the stage. Side reflectors scarcely meet the case. Writer has obtained best results by mounting a sub-stage mirror on a condenser stand, and using concave side to throw light from a lamp down on to slide ; but the stand has to be placed too close to microscope to be quite comfortable, and is apt to get shifted. A concave mirror working on a short bracket attached to limb carrying tube, on left hand of observer, in position to focus on stage, might be better.

Hints on Mounting.

Slides for Exhibition.—Small sporangia, on fragment of substratum, mounted dry in deep cells on 3 × 1 inch glass slips, are excellent objects for exhibition. When cell-rings are also of glass, more light gains access. Stalked sporangia should not be mounted vertically on slide, but horizontally or slantwise, so that whole length visible to observer. This can be done, if no other way, by fixing small ledge of cork to the slip, and mounting specimens on cork. A piece of paper of suitable tint for background—say very light green—can be pasted on back of slip behind cell, and scraped off when desired to look at specimen from other side. Such mounts must be perfectly dry before being sealed down, otherwise specimens may be spoilt.

Plasmodia.—Small plasmodia, or small portions of larger ones, will, under favourable conditions, spread over cover-glasses, and can then be fixed by alcohol, and treated with anilin, or other suitable stains, to show the multitudinous nuclei.

Spores.—Mount in glycerine jelly.

Capillitia.—Elaters and finer capillitia should also be mounted in glycerine jelly; but coarser capillitial structures need no medium, and can be mounted dry. After dispersion of spores, a sporangium of Stemonitis, dry - mounted, shows surface net extremely well under ½ inch objective. Spores can usually be got rid of by blowing sporangium not too violently.

Determination of Species.

In the Field.—If unfamiliar, the species found can generally be determined on the spot with approximate accuracy by using pocket-lens and referring to handbook mentioned.

By the Microscope.—To ascertain structural details on which precise identification depends, lay a sporangium on a glass slip, moisten with alcohol or methylated spirit to force out air from among spores, then add drop of water or water and glycerine. With a pair of mounted needles break up the capsule, gently shake out spores from capillitium, and use powers of microscope necessary to distinguish specific characters. The determining features are more fully described in the monograph.

V.

It is not, however, with the minutiæ of classification that the serious student will be chiefly concerned, nor will he haunt the woodlands in search of sporangia only. He will be far more interested in any plasmodium he may come across, in tracing its obscure procedure, and noticing the general effect upon the plasm of varying conditions of atmosphere, situation, and environment. Information of this kind can be gained only in the field, and the work of the future principally lies in this direction. ' When we are better acquainted with the conditions determining variations,' wrote the late Mr. Massee, ' it is certain that the main factor in the discrimination of species will not be a one-twelfth oil-immersion objective.'

From first to last Mycetozoa are anomalous and elusive. We classify them on the best basis we have as yet—on differences in stalks, sporangium walls, capillitia, and markings on threads and spore-cases; and then we reflect that, in themselves, all these things are of least importance—are, in fact, not living, but

dead things. There is no life in them. All the vitality is in the spore-plasm, and in that alone. The structures perish; only the spores survive, to regerminate wherever they find a home. There's a wind on the heath, and a breath in the woods; and spores—like human affairs—are the sport of circumstance.

> ' We are but thistle-globes on heaven's high gales,
> And whither blown, or when, or how, or why,
> Can choose us not at all.'

PLATE XI.

FIG. 130.—Germination of spores of Fungi and Mycetozoa :
  A. Germination of Fungus spores, the spores throwing out germ tubes × 390.
  B. Germination of spore of Mycetozoon, the swarm spore escaping from the spore case.
  C. Various forms of swarm spores of Mycetozoa × 500 to × 800.

FIG. 131.—Plasmodium of *Badhamia utricularis :*
  A. Extension of the creeping plasmodium spreading fanwise in a network of veins.
  B. General direction of currents of interior plasm flowing along the veins towards the advancing border.
  C. General direction of the retreating currents.

FIG. 132.—Development of sporangium of *Comatricha nigra* × 10.
  A. Cushion of watery plasm on surface of dead wood, from interior of which it has just emerged.
  B. Plasm more erect, with base of stalk visible at centre.
  C. Stalk lengthening upward. Plasm still adhering to base.
  D. Further extension of stalk. Plasm detached from base, and being drawn upward by rounding action of surface tension.
  E. Stalk at full length. Plasm rounded by surface tension. Interior secretions from plasm forming capillitium branching from summit of stalk. Capillitium partly visible. Sporangium becoming opaque.
  F. Final shape of sporangium. Spherical form modified by capillitium and effects of desiccation.

PLATE XI.
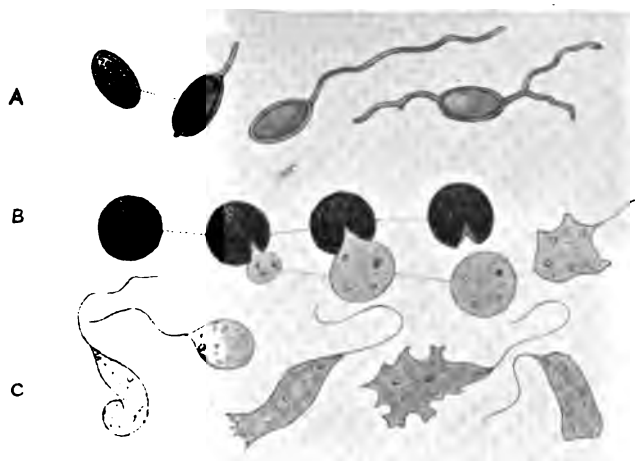
FIG. 130.
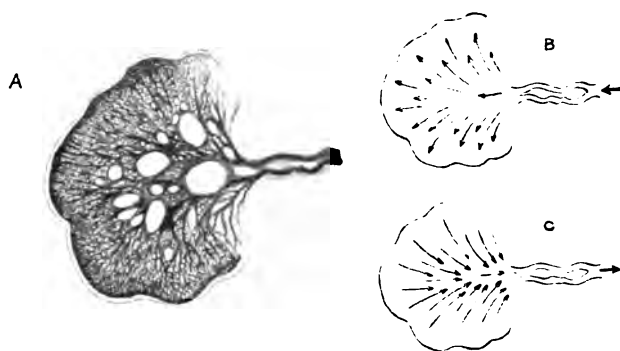
FIG. 131.
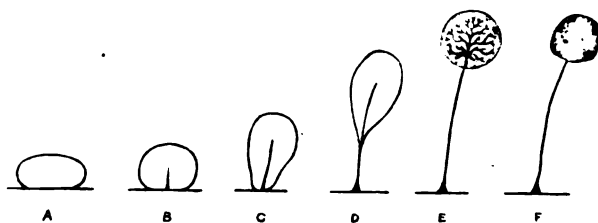
FIG. 132.
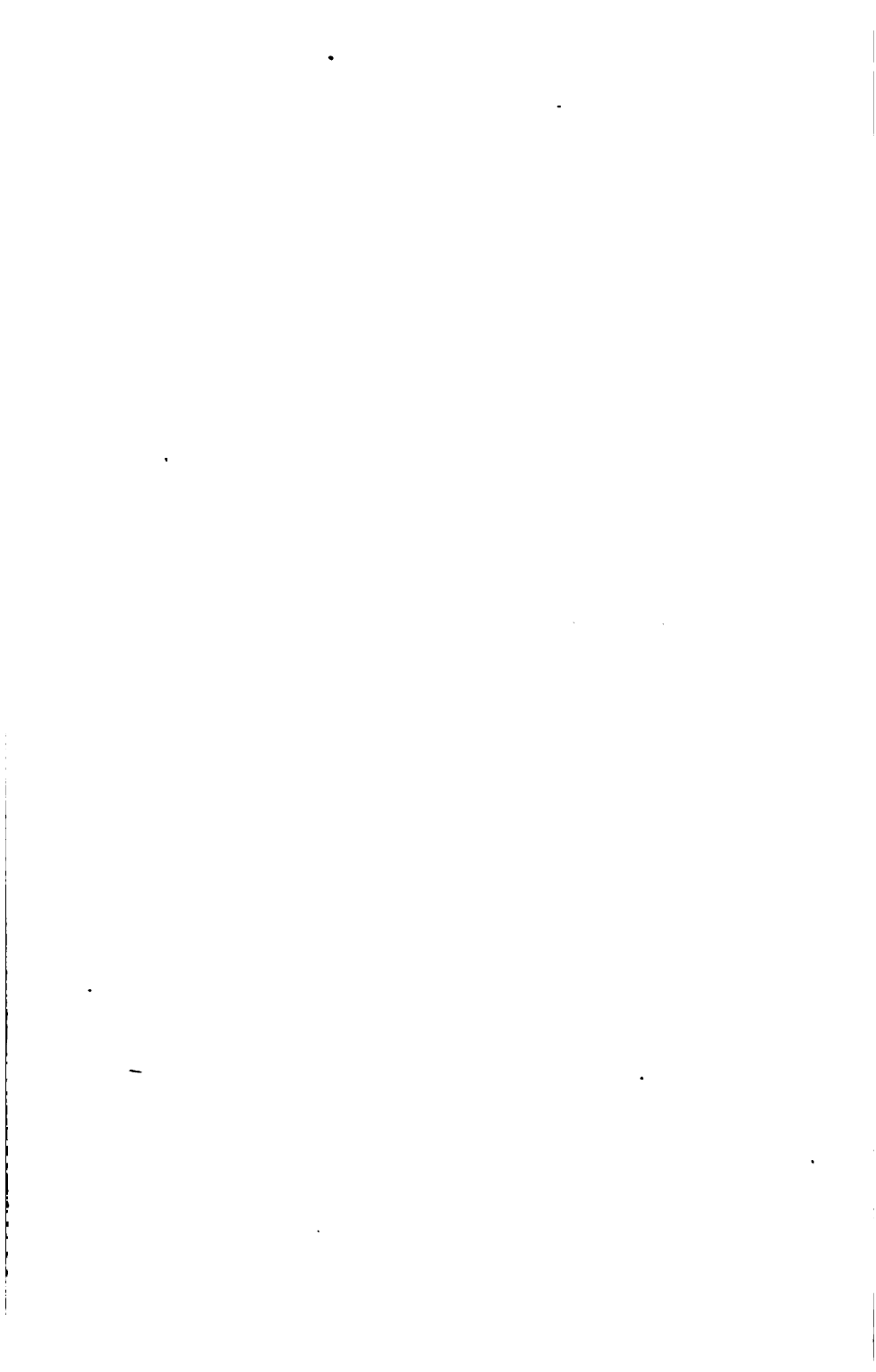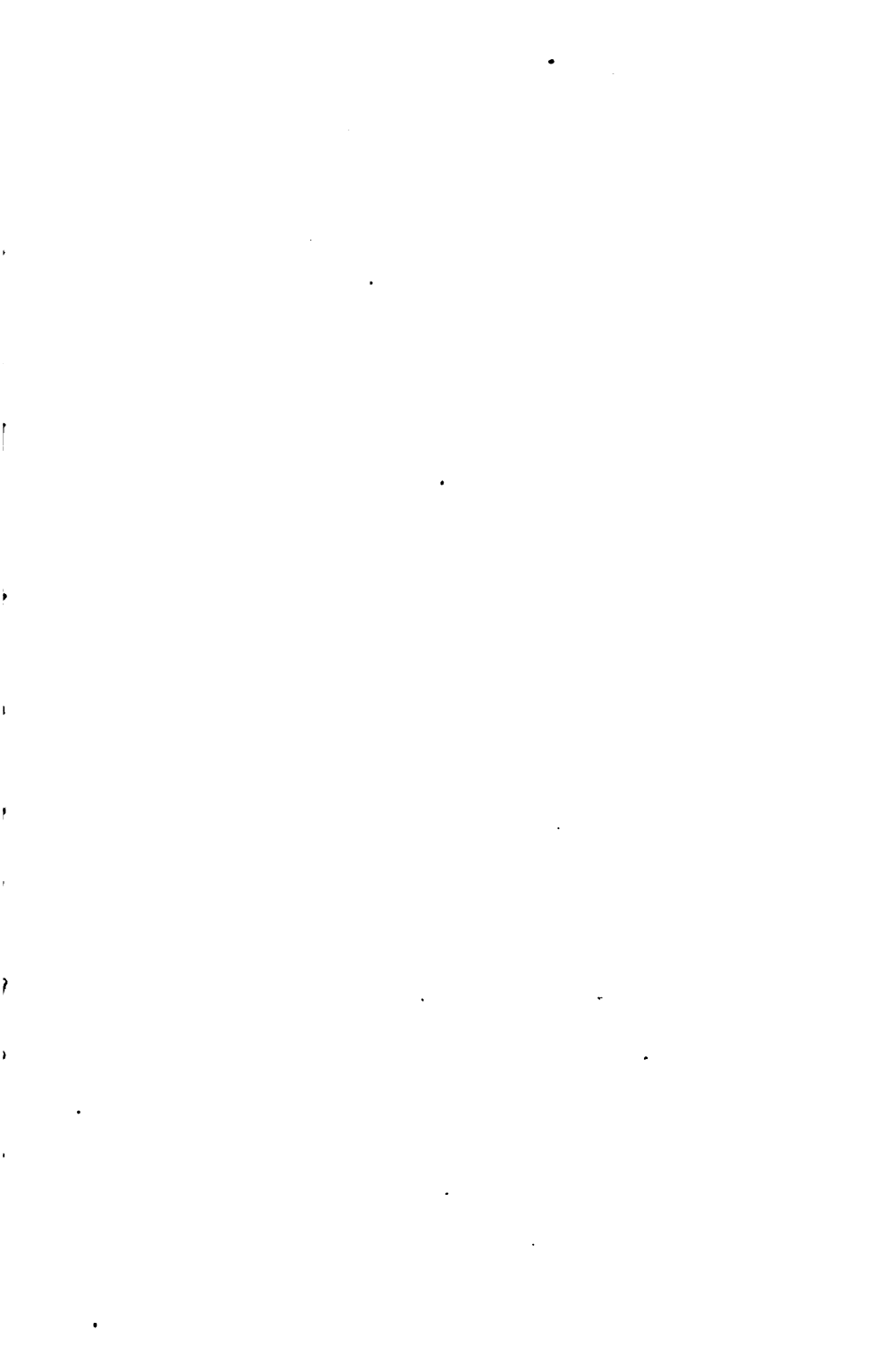
PLATE XII.

SPORANGIA OF MYCETOZOA FROM LIMETREE LOG.

A. E. Hilton del.

FIGS. 133-144.

PLATE XII.

Sporangia of Mycetozoa from Lime tree Log :

A. *Lamproderma columbinum :* Successive stages in development of sporangia × 3.

B. *Lamproderma columbinum :* Three sporangia, middle one with membrane flaking off, exposing spores, third one showing capillitium after dispersion of spores × 8.

C. *Lamproderma columbinum :* Sporangia, natural size.

D. *Arcyria denudata :* Patch of crowded sporangia, natural size.

E. *Arcyria denudata :* Three sporangia, two with expanded capillitium after dispersion of spores × 6.

F. *Fuligo septica :* Æthalium, one-third natural size.

G. *Lycogala epidendrum :* Æthalia, two-thirds natural size.

H. *Stemonitis ferruginea :* Group of sporangia × 1·3.

I. *Physarum nutans :* Two sporangia, one fractured, revealing spores × 12.

J. *Physarum nutans :* Group of sporangia × 4.

K. *Trichia scabra :* Cluster of crowded sporangia × 1·3.

L. *Perichæna corticalis :* Cluster of sporangia × 1·3.

MOUNTING COMMON OBJECTS *

By M. J. COLE.

Preparing and Mounting Entomological Specimens for the Microscope.

INSECTS should be killed with chloroform. They are then to be placed in methylated spirit, in which they may remain until required for mounting.

To Prepare a Whole Insect for Mounting with Pressure in Canada Balsam.—1. Transfer from methylated spirit to water, and let it soak for three or four hours to remove spirit.

2. Place in liq. potassæ—10 per cent. of caustic potash in distilled water—until soft. Some specimens will only require a few hours in the potash, others need days, and some even weeks, to soften. In all cases they must be carefully watched and the action of the potash tested. This can be ascertained by pressing on the thorax or chest of the insect with some blunt instrument such as the head of a pair of curved-pointed forceps.

3. When soft enough, pour away the potash and add water, which must be changed several times until all the potash is washed away.

4. Pour away the water and add concentrated acetic acid, and soak for twelve hours, or until it is convenient to go on with the work.

5. Transfer from acetic acid to water, and soak for about half an hour ; then place in a shallow saucer full of water, and with the aid of a needle and a camel's-hair brush spread out the wings, legs, etc. Now take a slide and place it in the water under the insect, lift the slide up carefully so that the insect may be stranded on the surface of the slide with all its parts

* *For further particulars as to Hardening, Embedding, Section cutting, Clearing, and Mounting, see Lessons I. to IV., Chapter XV.*

expanded. Drain off the excess of water, and lay the slide down on a piece of white paper, and with the aid of needles or brushes carefully place all the limbs, wings, antennæ, etc., in their natural positions. Now put a narrow slip of paper on each side of the insect, and carefully lay another slide over it, press it down until the insect is squeezed quite flat, tie the two slides together with a piece of twine, and place them in a jar of methylated spirit for at least twelve hours, or until required.

6. Remove the glasses from the spirit, carefully separate them, and with a soft camel's-hair brush push the insect off the glass into a saucer of spirit, and soak for half an hour.

7. Take the insect up on a lifter, and float it on to the surface of a small saucer of clove oil, and allow it to soak until perfectly clear.

8. Remove from clove oil and place in turpentine for a few minutes.

9. Mount in Canada balsam as directed for animal and botanical sections.

To Mount an Insect in Canada Balsam without Pressure. —Treat with potash as above, wash in water, and place in acetic acid. Wash away the acid with water, and transfer to a shallow saucer of methylated spirit. Take two needles and lay out the various parts as quickly as possible ; if any parts are troublesome, hold them in position until the spirit has fixed them. Now let it soak for an hour, or until required. Remove from spirit, place in clove oil, and when clear, place in turpentine.

Take a *tin* cell just deep enough for the specimen, and apply a coat of black shellac cement to one side of it. Allow this to nearly dry. Clean and warm a slide over a spirit-lamp; take up the cell in a pair of forceps, and bring the cemented side in contact with the centre of the warmed slide ; press on the upper side of the cell, until it adheres firmly to the slide, and put it away to dry. Fill the cell with Canada balsam, and see that it also flows over the upper edge of the cell, so that it may serve as a cement to fasten on the cover. Take the insect from the turpentine on a lifter, put it in the cell, and with needles re-arrange the parts if necessary. Put away out of reach of dust for twelve hours to harden the balsam. Place a drop of balsam on one side of the cell. Clean a cover-glass of the same size as

the cell, take it up in a pair of forceps, and warm it gently over a spirit-lamp, and bring its edge in contact with the drop of fresh balsam ; ease down carefully, so as to avoid air-bubbles, and press on surface of cover with a needle until it rests on the cell all round. Now take a soft brush and some benzole and wash away the exuded balsam ; dry with a clean rag, and apply a ring of cement.

To Mount an Insect in Glycerine without Pressure.— Many small, soft insects and their larvæ may be mounted in glycerine while fresh. The larger and harder kinds must be soaked in potash to render them transparent. Make a cell of the required size, and fasten it to a slide with black shellac cement, as directed for balsam mounts. Apply a coat of cement to the upper side of the cell. When cement is nearly dry fill the cell with glycerine, and put the insect into it ; spread out the wings, legs, etc. Clean and warm a cover-glass, and apply its edge to the cell ; press down, and be sure that it adheres to the cement all round. Wash away the excess of glycerine with some water, and dry the slide with a soft cloth. When quite dry, apply a ring of cement, and when this has dried, add another coat of black shellac cement.

Small insects, such as parasites, may be mounted whole in a cell in glycerine without treatment with potash, so that their internal organs may be seen *in situ*, but they usually require clearing. Take of Calvert's carbolic acid, solid at ordinary temperatures, 2 ounces, melt, and add about $\frac{1}{2}$ drachm of glycerine to prevent it becoming solid again. Soak the insect in this until transparent ; some specimens will only require an hour or two, others a week or more. When clear, make a cell as previously directed with any good shellac cement, and when dry, run on a coat of cement to its upper surface ; let this become about half dry, then place in the cell, fill it up with glycerine, and apply a cover-glass, which must be carefully pressed down with a needle-point until it adheres to the cement all round. The slide can then be washed with water to remove all trace of excess of glycerine ; put away until all the water has evaporated, then apply a coat of shellac cement, and when this has dried, rub away any water-marks that may be left on the slide with a soft cloth, and add another coat of cement.

Insect Dissection.—The processes described show only the external parts of the larger insects; all soft tissues and internal organs will, of course, have been destroyed by the potash. Soft internal organs must be dissected out of the specimen while under water.

Procure a gutta-percha dissecting-dish, lay the insect in it, and secure with pins in the desired position. If the abdominal or thoracic viscera are required, lay the insect on its back; if the nervous system, on its ventral surface. Fill the dish with water, and with a pair of sharp-pointed scissors cut through the chitinous skin on each side of the abdomen, taking care not to cut too deeply so as to injure the internal organs; then with a pair of forceps raise and remove the skin. The organs may now be removed with the aid of a pocket-lens, and washed in distilled water; then stain in borax carmine for several minutes, wash in methylated spirit; then immerse in acidulated alcohol for a few minutes, dehydrate, clear in clove oil, and mount in Canada balsam.

If it is desired to mount the specimen in glycerine, stain as above, wash away all trace of spirit with water, and mount in glycerine jelly; if the specimen requires a cell, it must be mounted in glycerine.

Salivary glands of cockroaches and crickets, gizzards of beetles, and stings of bees and wasps, may be easily removed in the following way: Place the specimen whole and while quite fresh in water, cover with a piece of paper or anything to keep out dust, and let them soak for several days until the smell becomes rather unpleasant; then wash in clean water, hold the insect between the fingers, and with a pair of forceps carefully pull off the head, which should bring with it the œsophagus, salivary glands, and stomach. For stings of wasps and bees proceed as follows: Gently squeeze the abdomen of the specimen between the fingers of the left hand until the sting protrudes, then grip it with a pair of fine forceps, and gently pull it out. If properly done, the poison gland and duct should come away with it. Wash in water, and place it on a slide under a dissecting microscope, and with a fine needle-point draw the stings from their sheath; this is done by putting the needle under the stings at the base of the sheath and carefully drawing it towards the apex.

Stain in borax carmine, wash in alcohol, then in acidulated alcohol, and place in water; now lay out on a slide, place another slide over it, tie with thread, and immerse in methylated spirit for several hours; remove from glass, clear in clove oil, and mount in Canada balsam.

Wing-cases, legs, heads, and feet of diamond beetles should be mounted in opaque cells in Canada balsam. Take a slide, and with a turn-table run on a disc of black varnish of the required size; allow this to dry thoroughly. Take a piece of black gummed paper and punch out a disc of the same size as that on the slide to which it is to be fastened. Now take a *tin* cell of the required depth—on no account use brass or vulcanite cells; they are affected by the balsam, and the mount will be spoiled—lay the cell on a slide, and apply a coat of cement to its upper surface; allow this to become nearly dry, then take up the cell in a pair of forceps, and bring its cemented surface in contact with the paper disc on the slide, and with the point of the forceps press the cell down until the cement adheres to the paper. Now put away to dry in some place protected from dust. Take the specimen to be mounted, examine it under a microscope, and if dirty wash in some benzole, and then let it dry again. Now place a small quantity of gum-water in the centre of the cell, and put the specimen into it in the desired position; make sure that it adheres securely to the gum, and put the slide away again until everything is quite dry. Put the slide in a turn-table, and run on a coat of shellac cement to its upper surface, and allow it to become nearly dry; then fill up the cell with Canada balsam, clean, and apply a cover-glass, which must be well pressed into the cement until it adheres firmly; put away for an hour, and then wash away the exuded balsam with a soft brush and some turpentine; dry the slide with a soft rag, and apply a coat of black shellac cement.

Heads of flies having coloured compound eyes, such as Tabanus, lace-wing flies, etc., should be mounted in opaque cells in glycerine. Make the cell in exactly the same way as directed for balsam mounts, but take care that the cell is only just deep enough to take the specimen, as the object has to be retained in the centre of the cell by slight pressure on the part of the cover-glass. When the cell is quite dry, apply a coat of shellac cement

to its upper surface, and let it nearly dry; then take a brush and some clean water and moisten the inside of the cell. This is done to prevent the formation of air-bubbles, for if glycerine is put into a dry cell, bubbles are sure to give a lot of trouble. Now fill the cell with glycerine and put in the specimen, which should be previously soaked in dilute glycerine for an hour or two, and with a needle place it in the desired position; apply the cover-glass very carefully, so that no air-bubbles may be enclosed, and let it settle down by its own weight until it rests on the surface of the cell; then press it down with a needle-point until securely embedded in the half-dried cement, and set aside for an hour or two to dry. The exuded glycerine may then be washed away by holding the slide under a water-tap. When all trace of glycerine is removed, dry the slide with a soft cloth, and apply a coat of black shellac enamel.

Heads of large insects may be secured in the centre of the cell in the following way: Take a fine needle, thread it with a hair, and run it through the specimen. Unthread the needle, take up each end of the hair with the object suspended and stretch it across the cell so that it may be embedded in the cement on each side. Now apply a cover-glass, press it down until securely fixed, and if the specimen is not in the middle of the cell, adjust it by pulling on the hair on one side. Put away to dry, cut off the ends of the hair close to the edge of the cell, wash away excess of glycerine, dry, and apply a coat of shellac enamel.

Crystals and Polariscope Objects.

Crystals.—*Method* 1.—Make a strong solution of the material in distilled water, with the aid of heat if necessary, and filter; take up a small quantity of the solution in a dipping-tube, and drop it on a cover-glass. Prepare several covers in this way, and allow some to dry slowly, and evaporate others over a spirit-lamp. When dry, add a drop or two of Canada balsam, and mount in the usual way.

Method 2.—Make a strong solution in distilled water, and add a few drops of gum water or a small piece of gelatine; mix well, and filter. Apply some of the solution to a cover-glass, and allow it to dry slowly in a place protected from dust. Mount in Canada balsam.

Method 3.—Place a small piece of the dry crystal on a slide, and apply a cover-glass; warm over a spirit-lamp until fusion results, press the cover down with a needle, and allow the slide to cool. Clean off the exuded material, and finish off with some good cement.

Some crystals are soluble in Canada balsam; in which case, mount in castor oil.

Crystallize the specimen on the cover-glass; make a thin cell with some shellac cement on a slide, and allow it to become perfectly dry; then apply another coat of cement, and when this has nearly dried, fill the cell with castor oil. Take up the cover with a pair of forceps, and bring the crystallized surface in contact with the oil, being very careful that no air-bubbles form. Ease it down gently, and when it rests on the cell, give it a press with the point of the forceps; this will squeeze out the excess of oil and embed the edge of the cover in the cement. Put away to dry; wash off the exuded oil with some turpentine, and apply another coat of shellac cement.

The following salts, etc., are easily obtained, and they all give very good results:

Chloride of barium.*	Sulphate of iron.*	Asparagine.
Chlorate of potash.*	Tartrate of soda.*	Quinidine.
Sulphate of copper.*	Salicine.	Santonine.
Spermaceti (fuse).	Stearine (fuse).	Tartaric acid.

Those marked * are more effective when crystallized in gum or gelatine.

Crystals of Silver.—Clean a cover-glass and fasten it to a slide with the breath; make a 1 per cent. solution of nitrate of silver, and place a drop of it in the centre of the cover-glass. Now add a very small fragment of copper, and put the slide away out of reach of dust until the crystals have formed and all moisture has evaporated. Then make a shallow opaque cell, and place a small drop of gum water in its centre. Take up the cover with a pair of forceps—crystals uppermost, of course—and drop it into the cell; now take a needle-point, and carefully press on the cover-glass between the crystals, until it lies quite flat, and air-bubbles, if any, have exuded. Put the slide away again until the gum has dried. Now put the slide into a turn-

table; run on a coat of shellac cement to the upper surface of the cell. Allow this to become half dry, and then apply a cover-glass.

The following specimens from the vegetable kingdom make fine polariscope objects: Starches, hairs, scales from leaves, cotton and silk fibres, cuticles of leaves, and longitudinal and transverse sections of stems.

Starches can be obtained from most vegetable substances by scraping the cut surface with a knife. Place the scrapings in a bottle of water and shake well; then strain through muslin of sufficiently fine texture to allow the starch to pass, but to retain the fibres. Now put the strained material into a bottle, shake it up, and then allow to settle; the starch will fall to the bottom of the bottle in a few minutes. Then pour off the water; add some more, and repeat the process until all trace of cellular tissue is removed. When the starch is quite clean, take up a little in a dipping-tube; apply a drop to a clean cover. See that it spreads evenly all over the surface of the cover, and put away, protected from dust, until quite dry; then add a drop of Canada balsam, and mount in the ordinary way.

Starches may also be mounted in glycerine jelly (see p. 158), but they do not polarize so well as the balsam preparations.

Sections of Starch-Bearing Tissues.—The stems, roots, and bulbs must be hardened in methylated spirit for a week; then make transverse or longitudinal sections. Dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Cuticles containing Raphides.—The most common are taken from the following bulbs: garlic, onion, lily, hyacinth. Strip off the cuticle from the fresh specimen; dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Cuticles of Leaves.—Cut up the leaf into small pieces, and soak in water until rotten; the cuticles can then be separated, washed in water, dehydrated in methylated spirit, cleared in clove oil, and mounted in Canada balsam.

Cotton, Hemp, Wool, Silk, Flax, etc.—Place the fibres in methylated spirit to dehydrate; then clear in clove oil, and place a little on a slide. Separate the fibres from each other with needle-points; apply a few drops of Canada balsam and a cover-glass.

Scales of Leaves.—Scrape the leaf with a knife, and put the scrapings into a bottle of turpentine, and soak until all trace of air has disappeared from the scales; then pour off the turpentine. Take up a little of the scales on the point of a penknife, and mount them in Canada balsam in the ordinary way. Some leaf-scales are very difficult to deprive of air; in fact, it is impossible to get them quite free.

The following animal tissues make good polariscope objects: fish-scales, palates of mollusca, sections of hairs and quills, horns and hoofs, whalebone, claws of dogs, cats, and fowls, decalcified bones, muscular tissues.

Fish Scales.—Scrape the fish from the head towards the tail; if scraped the other way, nearly all the scales will be injured. Place the scrapings in a bottle of water, shake well, pour off the water, and repeat the process until quite clean. Examine with a microscope, and if you find that the scales are not clean, pour off the water, add liq. potassæ, and soak for an hour or two; then wash away the potash with repeated changes of water, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Sometimes fish scales buckle up in spirit, and they will not lie flat. When this happens, put them into water again, and soak a little while; then place them on a slide, and put another slide over them, press down until quite flat, and tie the two glasses together with twine, and place them in a vessel of methylated spirit to dehydrate under pressure. This method will answer for all tissues that have a tendency to twist during the process of dehydration.

Palates.—Dissect out, and soak in liq. potassæ for a few days. Wash well in water, spread out on a slide; put a piece of paper on each side of it to prevent crushing, and place another slide over all in the same way as directed for insects; tie the glasses together with string, and place in methylated spirit for an hour or two. Then remove the palate from the glasses, and place it in clove oil until clear. Mount in Canada balsam.

Sometimes it is very difficult to dissect out the palates from small snails. This process answers just as well: Cut off the head of the animal, being careful that you remove the buccal mass with it, and place in liq. potassæ for a few days; this will

destroy all the soft tissues, but not the palate or radula. Wash away the potash with repeated changes of water, and proceed as directed above.

Sections of Hairs and Quills may sometimes be cut after soaking for a few days in methylated spirit; but some of the larger kinds, such as the whisker of walrus, will require softening in potash. Place in liq. potassæ for a few hours or days, in accordance with the consistency of the tissue. When soft enough, wash away the potash with water, and place in methylated spirit, in which they may be preserved until required. Then make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Small Fine Hairs.—Cut off a number of hairs, tie them up into a bundle with some cotton, and soak for a few minutes in warm water. Make up a strong solution of gelatine in water, and transfer the bundle of hairs to it, and soak it for several hours in a hot-water bath until the gelatine has penetrated to the centre of the bundle. Remove from the gelatine on the point of a needle, and hold it exposed to the air until the gelatine has cooled; then push them from off the needle into a bottle of methylated spirit, and soak for an hour or two to complete the hardening. Embed in carrot, put in a microtome, and cut transverse sections, and as they are cut place them in methylated spirit to dehydrate; then clear in clove oil, and mount in Canada balsam.

Horns, Hoofs, Whalebone, and Claws all require steeping in liq. potassæ until soft; they are then to be washed in water, and preserved in methylated spirit until required. Embed in carrot, place in a well microtome, make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Decalcified Bones (see Chapter XV., p. 123).—Embed in carrot, make transverse and longitudinal sections, dehydrate in methylated spirit, clear in clove oil, and mount in Canada balsam.

Muscular Fibres.—Take the tongue of a cat, harden it in methylated spirit for a week or ten days; then embed in carrot, and make transverse or longitudinal sections, dehydrate, clear in clove oil, and mount in Canada balsam.

Cleaning and Mounting Diatoms, Polycystina, etc.*

To Clean Diatoms growing upon Algæ or Shells.—Place the algæ or shells in a basin, cover them with water, add hydrochloric acid, and stir until effervescence results; add more acid little by little, until effervescence ceases, stirring from time to time. Now strain through net of sufficiently fine texture to allow the diatoms to pass, but to retain the débris. Allow the strained fluid to settle down, pour off the acid water, and place the deposit in a large test-tube. Add pure hydrochloric acid, and boil for twenty minutes; add some pure nitric acid, and boil again for twenty minutes, and, while boiling, add some crystals of chlorate of potash until complete bleaching results. Remove all trace of acid or alkali by washing in water, and examine the forms under the microscope. If clean, bottle them up in distilled water for future mounting. If, as is sometimes the case, there has been animal matter present which has not been removed, boil in pure sulphuric acid for a few minutes. Wash away all trace of acid before bottling the diatoms in distilled water.

To Clean Fossil Diatomaceous Deposits. — Break the deposit up into small pieces, and place them in a large test-tube in a moderately strong solution of bicarbonate of soda, and boil gently for two hours, the disintegrated portions being from time to time poured off into a beaker and the boiling in soda continued until all the deposit has broken up. The alkaline solution must then be washed away, and the diatoms boiled for a short time in nitric acid, and when sufficiently clean wash away the acid in repeated changes of water, and bottle up the diatoms in distilled water.

To Clean Living Diatoms.—Remove all dirt or salt by washing well in water; shake well, and allow the diatoms to settle before pouring off the water. In this way all soluble impurities can be removed. When the water remains clear, pour it off, leaving the diatoms as nearly dry as possible, and cover them with strong alcohol, which will extract the endochrome; change the alcohol daily until it ceases to be tinged with green; then wash away the alcohol with water, pour off the water, and place

* To clean and mount Foraminifera see Chapter XXI.

the diatoms in a platinum capsule and heat them to a dull red over a spirit-lamp. This will separate the frustules into single valves, and finish the cleaning of the diatoms, and they may then be bottled up in distilled water.

To Clean Polycystina.—The polycystinous earth should be broken into small pieces and boiled for several hours in a strong solution of common washing soda, the disintegrated matter being from time to time poured off into a vessel, and the boiling in soda continued until all the earth is broken up. Wash the disintegrated matter in water several times to remove the soda, allow the polycystina to settle down, and pour off the water and place the forms in a test-tube; add some nitric acid, and boil for twenty minutes. Remove all trace of acid with water, and bottle up in distilled water.

To Mount Diatoms in Canada Balsam (*Unselected Slides*). —The diatoms are to be taken out of the bottle with a dipping-tube, and should be allowed to fall upon a clean cover-glass. The fall of the drop causes the forms to spread evenly over the cover. It should then be dried slowly over a spirit-lamp. When dry a small drop of Canada balsam is to be applied, and the slide put away out of reach of dust to dry for twelve hours. Now place on a hot plate, and apply gentle heat from a spirit-lamp for about ten minutes. Allow it to cool. Take the cover up with a pair of forceps, and bring its balsamed surface in contact with the centre of a warmed slide. The balsam should then run to a neat bevelled edge all round the cover; should it not do so, warm the slide a little more until it does.

Unselected Polycystina.—Take the forms from the bottle with a glass tube, and spread them on a slide; dry them over a spirit-lamp. Now clean a cover-glass, fasten it to a slip with your breath, and place a drop or two of balsam on it; take up some of the polycystina on the point of a knife and place them in the balsam; stir them well up with a needle and put away for twelve hours. Bake over a spirit-lamp for ten minutes, and while warm stir up again gently with a needle, and spread the forms evenly over the cover. Warm a glass slide, and proceed as directed for unselected diatoms.

Unselected Polycystina as Opaque Objects.—Dry some polycystina on a slide, then take a platinum capsule, put the

dried material into it, and heat over a spirit-lamp to a dull red. Clean a cover-glass, fasten it to a slide with your breath, and apply a few drops of balsam. Take up some of the dried forms, put them into the balsam, and stir up with a needle until they are evenly spread over the cover; put away out of reach of dust for twelve hours, so that the air may escape from the forms. Now place on a hot plate and apply gentle heat for ten to fifteen minutes to bake the balsam. Clean another cover-glass, add a drop or two of balsam to the hardened balsam, and apply the second cover-glass; warm again, and with a needle press gently on the upper cover until it lies perfectly flat; then allow to cool, apply a coat of black shellac cement all over one side of the upper cover, and put away to dry. In the meantime take a slip, put it in a turn-table, and run on a disc of black varnish of the same size as the cover; let this dry, then add a drop of strong gum or glue; take up the covers with a pair of forceps, and put the blackened side into the glue; press down with a needle until the glue spreads evenly under the cover, and put away to dry. When dry, finish off with a coat of black cement.

Selected Diatoms and Polycystina.—Take an ounce of distilled water, add 6 or 8 drops of ordinary gum water, and filter. Clean a cover-glass, and place a drop of the diluted gum upon it; put away to dry.

Spread the diatoms or polycystina on a slip, and dry them over a spirit-lamp. Select the desired forms with a fine brush or bristle, and breathe upon the gummed surface of the cover, and place the forms upon it. When dry, apply a drop of balsam, and put away out of reach of dust for twelve hours. Bake and finish as directed for unselected slides.

In mounting selected polycystina, they must be between two covers; if on a single cover, the forms would be upside down when the cover was reversed. If a transparent mount is desired, the two covers can be fastened to the slide with a drop of balsam. If opaque, the forms must be burnt, and one side must be blackened; in other respects proceed exactly as you would for unselected opaque mounts.

Polycystina may also be mounted in a dry opaque cell. Take a slide, run on a disc of black varnish, and when this has dried fasten a disc of black gummed paper over it. Then take a

shallow cell, apply a coat of cement to one side of it, and let it nearly dry; then fasten to the paper disc, and put away to dry. Apply a little dilute gum water to the bottom of the cell, select the specimens, and put them into the gum; if they do not adhere, breathe on the surface of the gum. When all are arranged, put the slide away until everything is quite dry; then add a coat of cement to the upper side of the cell, let it nearly dry, and then apply the cover-glass.

Spicules of Gorgonia or Sea-Fan.—Boil in liq. potassæ until all the material has broken up, then wash away the potash with repeated changes of water, allowing the spicules to settle to the bottom of the tube between each washing. When cleaned, preserve in a bottle of dilute spirit. Proceed with the mounting in exactly the same way as directed for transparent unselected polycystina.

Spicules of Alcionium.—Proceed as above.

Sponges.

1. To Show Cell Structure, Flagellated Cell, etc.—Fresh specimens of the calcareous forms—Sycon, for example—should be fixed with osmic acid 1 per cent. solution, washed in distilled water, and placed in absolute alcohol for twelve hours; then soak in absolute alcohol and ether for a few hours, infiltrate, and embed in celloidin. Cut sections in a microtome. Place sections in absolute alcohol for about three minutes, clear in oil of origanum, and mount in Canada balsam.

If preferred, sections of sponges may be mounted in glycerine jelly, but they must be soaked in water for a little while before they can go into the jelly.

2. The Skeleton—(*a*) *Horny Sponges.*—Boil in liq. potassæ, then wash the spicules well in water, and mount in glycerine jelly or Canada balsam.

(*b*) *Calcareous.*—Dehydrate small forms in alcohol, clear in clove oil, and mount in Canada balsam in a cell; or separate the spicules by boiling in liq. potassæ, wash in water, and mount in Canada balsam or glycerine jelly.

(*c*) *Silicious.*—Boil in nitric acid, wash well in water, dehydrate, clear, and mount in Canada balsam.

For the types in which silicious spicules are embedded in

horny material, boil in liq. potassæ for a few minutes to disintegrate the tissues, then in nitric acid to clean the spicules, wash well in water, and mount in Canada balsam.

Sections of Sponges.—Harden in methylated spirit, and transfer to equal parts of ether and absolute alcohol for several hours. Then place in a thin solution of celloidin for a day or two, transfer to a thicker solution of celloidin, and soak again for a few hours. Remove from the celloidin on the point of a needle, and hold exposed to the air for a few minutes to allow the celloidin to set around the specimen; then push it off the needle into a bottle of methylated spirit, and soak for a few hours to complete the hardening. Embed in carrot, place in a well microtome, and make the sections. Dehydrate in methylated spirit, clear in oil of bergamot, and mount in Canada balsam.

Sometimes sponge sections are rendered too transparent by mounting in balsam. In such cases, mount in glycerine jelly, but be careful to wash away all traces of alcohol before they go into the jelly.

Cutting, Grinding, and Mounting Hard Substances.

Rock Sections.—Small pieces or slices of rock are to be ground on a zinc plate with the aid of emery powder and water until one side is quite flat and smooth. Then fasten the polished surface to a square of glass with some dried Canada balsam, as directed for bone (see p. 147). When cool grind the other side on the zinc plate with coarse emery and plenty of water. When moderately thin, take a piece of plate-glass and some fine flour-emery, and rub the section down as thinly as possible. When thin enough, wash well in water and dry; then warm over a spirit-lamp, and with a needle push the section off the glass into a saucer of benzole or turpentine, and allow it to soak until all the balsam is dissolved. Wash again in some clean benzole, and mount in Canada balsam in the usual way. Sections of echinus spines, shells, and stones of fruit are prepared in the same way as bones and teeth; but when the grinding is finished, the sections are to be passed through alcohol into clove oil, then mount in Canada balsam in the usual way.

Sections of coal containing fossils, limestone, spines of echinus,

and other friable specimens should be cut with a very fine saw, and then soaked in benzole for several hours. When the benzole has saturated the tissue, transfer to ordinary solution of Canada balsam in benzole, and soak again until the balsam has penetrated to the centre. Take a 8 × 1 inch slide, place the section on its centre, and add sufficient balsam to cover it. Put away out of reach of dust until the benzole has evaporated from the balsam. Then place on a hot plate, apply gentle heat with a spirit-lamp, and bake until the balsam is quite hard. Grind down to the required thinness on a hone. Wash well with water, dry, add a few drops of fluid balsam in benzole, and apply a cover-glass.

Staining and Mounting Micro-Organisms.

The investigation of bacteria may be carried out under various conditions :

(1) In fluids, such as milk, water, blood, pus, etc. (2) On solid media, bread, meat, potatoes, meat jelly, etc., or in the tissues and organs of animals. In the former case a drop of fluid is placed on the centre of a cover-glass, and another cover-glass is placed on it; the two glasses are then to be rubbed together to spread the organisms evenly over their surfaces; they are then separated and allowed to dry. When bacteria are growing on solid material, scrape off a small portion, put on a cover-glass, and treat as above; separate the covers, and allow to dry. When the cover is quite dry, take it up with a pair of forceps, organisms uppermost, and pass two or three times through the flame of a spirit-lamp; this will fix the albumen and fasten the bacteria to the glass.

To Stain Bacteria on Cover-Glasses.—They should be floated with the organisms downwards on a saturated watery solution of any of the following aniline dyes: Methyl blue, methyl violet, gentian violet, fuchsin, vesuvin, or Bismarck brown. From ten to fifteen minutes is enough for the first four stains; vesuvin and Bismarck brown require about an hour. When the staining is complete wash the cover in distilled water. If the colour is too deep wash it in a ½ per cent. solution of acetic acid, and then again in water; put away to dry. When quite dry add a drop of Canada balsam, and mount on a slide in the usual way.

When bacteria are present in the organs of animals the tissues should be hardened in methylated spirit for about a week, and very thin sections with a freezing microtome cut from them. The sections may be stained in any of the above dyes; then wash in water, dehydrate in absolute alcohol, clear in oil of cedar or bergamot, and mount in balsam.

Staining Non-Cellular Structures.

There are some objects which do not present any true cellular structure, and are consequently refractory to ordinary staining methods.

The radulæ of mollusca come under this category. Good results may be obtained, however, by the method introduced by Dr. Bowell, as follows : Wash in dilute acetic acid, after cleaning with caustic alkali, then warm them in a 1 per cent. solution of potassium permanganate over a spirit lamp (a watch-glass is a convenient vessel), adding to the solution immediately before use a few drops of strong acetic acid. The solution will turn brown and precipitate, and the radula will become black. Now add one or two small crystals of potassium oxalate. The solution will clear, leaving the radula bleached. After rinsing with water, it may now be stained in an aqueous solution of dahlia (or other basic dyestuff). The stain is differentiated, and the object mounted in Canada balsam in the usual way.

Dry Mounts.

Opaque Cells.—Place a slide in a turn-table, and run a disc of black varnish on its centre : allow this to dry. Take a piece of black paper and punch out a disc of the same size as the one on the slide, and gum it on to the varnish spot. Take a cell, either metal or vulcanite, of the required depth and fasten it to the paper disc with gold size or black shellac cement, and put the slide away until quite dry. Now place a very small quantity of gum on the centre of the paper disc, and put the specimen into it; but take care that the gum does not extend beyond the object, or the appearance of the mount will be spoiled. When the gum has dried, put the slide into the turn-table again, and run a ring of any good cement on the upper surface of the cell, and when this has become about half dry apply a cover-glass.

which must be pressed down with a needle-point until it adheres firmly to the cement all round the cell. Put the slide aside for an hour or two, and then run on a good coat of black shellac cement.

Feathers of humming-birds, eggs of butterflies and moths, small microscopic seeds, gills of many fishes, skins of fishes, skins of snakes, and transverse or longitudinal sections of stems of plants, are all mounted as opaque objects in the same manner as above. The former should be arranged in the cell in a group. The gills, skins, etc., should be well washed with distilled water and dried under pressure between two glass slips tied together with twine.

Transparent Cells.—Take a cell of the desired depth and apply a coat of cement to one side of it, and allow it to become very nearly dry. Take a slide and warm it gently over a spirit-lamp; take up the cell with a pair of forceps and place it on the centre of the slide, the warmth of which should cause the cement of the cell to melt; if not, warm a little more, and press the cell down gently with a needle-point until it adheres firmly to the slide all round. If the specimen is small it must be fastened in the cell with some gum, as for opaque mounts, then put it away until the gum has dried, apply a cover, and finish off as directed for opaque mounts. Leaves of plants and wings of butterflies should be mounted on a thin slide, so that both sides may be examined. No gum will be required for these specimens, but a piece of the leaf or wing should be cut or punched out as nearly the size of the cell as possible, and a thin cell should be used, so that the cover may rest on the object and keep it flat. In all dry mounts great care must be taken that all the cements used to fasten the objects in position are quite dry before the cover is put on; if not, any moisture remaining will condense on the under surface of the cover and spoil the preparation.

Opaque Mounts of Pollens.—Make an opaque cell, and apply a thin layer of gum water all over its floor; then take some perfectly dry pollen and put it in the cell, shake the slide so that the pollen spreads evenly all over the cell, and let it dry. Then apply some enamel to the upper surface of the ring of the cell, and when this is about half dry apply the cover-glass.

Finishing off Slides.

Canada Balsam—*Quick Method.*—Take a small saucer of chloroform and a soft brush, and carefully wash away the exuded balsam. Allow the slide to dry, then place it in a turn-table and apply a coat of black shellac cement. Let this dry, then wash the slide quite clean with turpentine and apply another coat of cement.

Canada Balsam—*Exposure Method.*—Put the slide into a saucer of methylated spirit, and with a small piece of soft rag gently rub away the excess of balsam; dry the slide with a clean cloth, and apply a coat of any good cement.

Glycerine Jelly.—Put the slide in a saucer of cold water and allow it to soak for a few minutes, then take a penknife and carefully scrape away the jelly from the edge of the cover. Give the slide a good wash in water, and place it in some methylated spirit, which will remove the water. Dry with a clean soft cloth, and apply a coat of black shellac enamel, and when this has dried add another.

Farrant's Medium.—Allow the slide to dry for a few days, then put it into a saucer of water and wash away the excess of medium with a soft brush. Drain off as much water as possible, and, if the cover is firm enough, dry the slide carefully with a soft cloth; if not, allow all the moisture to evaporate by exposure to the air. When quite dry, put it in a turn-table and apply a coat of cement, and when this has dried add another.

Dry Mounts do not require any washing, but they should have one or two coats of any good cement.

Asphalte and white zinc cement may be used when desired for balsam or dry mounts, but they are both useless for any of the aqueous or fluid media.

A really good black enamel may be made in the following way:

Dissolve best black sealing-wax in methylated spirit until the solution is as thick as treacle, then mix this with an equal quantity of marine glue; then if too thick, dilute with a little methylated spirit. This cement has been alluded to in these chapters as shellac cement, and it is the best I know of for general purposes. The black enamel should be kept in a wide-

mouthed, stoppered bottle.   Should the stopper become fixed, just warm the neck of the bottle over a spirit-lamp; it can then be easily removed.

When a ring is being applied to a slide, the turn-table should not be run too fast, and the extreme point of the brush should only just touch the glass.   A thin coat must be run on at first, then give it about ten minutes to dry.   A sufficient quantity of cement may then be added to finish the mount, but if too much is applied at first it will overflow.

The most suitable brush for ringing slides is a sable 'rigger' No. 2 in a metal holder; it should be well washed in methylated spirit after use.

Cleaning off Failures.—During a course of microscopical work many slides will be not worth keeping, but the slips and covers are quite good, and they can be used again.   When a batch of failures has accumulated, make a strong solution of Hudson's soap-powder in warm water, and place some of it in two jars. Warm the slide over a spirit-lamp, and with a needle-point push off the cover into one of the jars and put the slip into the other; let them soak for an hour or two, then wash away the soap solution with repeated changes of warm water, and finally pour away all the water and add methylated spirit; soak for a little while, and then dry with a soft clean rag.

Sometimes slips and covers have a dull, cloudy appearance, which defies all attempts to remove it.   When this is the case, make up a solution of hydrochloric acid in methylated spirit (about one part of acid in six of spirit), and immerse the glasses for a few minutes.   Wash away the acid with methylated spirit, and dry with a soft rag.

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